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The Spectrum of Uses for Growth Hormone in Children

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INTRODUCTION

This is a reasonable time for a reassessment of the use of growth hormone (GH) in childhood, as pituitary-derived human GH (hGH) was first tested in children 40 years ago and recombinant DNA-derived hGH has been available for more than a dozen years. Multiple controlled and uncontrolled clinical trials have been performed and tens of thousands of children worldwide are receiving therapy. GH has received Food and Drug Administration (FDA) approval in the United States for 3 pediatric indications: childhood GH deficiency (GHD), growth failure associated with chronic renal insufficiency (CRI); and short stature associated with Turner syndrome (TS). Treatment also has been approved for AIDS-associated wasting and adult GHD. The ability to produce recombinant GH in essentially unlimited quantities has allowed higher doses to be employed for both conventional and less conventional uses. Provocative GH testing, for years the definitive diagnostic method for GHD, has come under renewed criticism; alternative diagnostic strategies, such as 24-hour GH sampling, quantitative excretion of urinary GH, and determinations of serum concentrations of insulin-like growth factor 1 (IGF-1), IGF-binding protein 3 (IGFBP-3) and, possibly, the acid labile subunit (ALS), have been proposed. Although Creutzfeldt-Jakob disease is not a complication of

In This Issue

The Spectrum of Uses for Growth Hormone in Children ..... page 1
Letter From the Editor ..... page 1
How Safe and Effective Is Human Growth Hormone at Pharmacologic Dosing? ..... page 4
CME Information ..... page 5
Abstracts ..... page 8

Letter From the Editor

The two lead articles in this issue are published primarily for nonendocrinologists who read *GROWTH, Genetics, & Hormones*, although endocrinologists also will undoubtedly appreciate the authors' opinions. The purpose of these articles is stated in the opening sentence of Dr. Rosenfeld in his article entitled "The Spectrum of Uses for Growth Hormone in Children." Specifically, Dr. Rosenfeld states: "This is a reasonable time for a reassessment of the use of growth hormone (GH) in childhood." Dr. Rosenfeld is eminently qualified as an expert both in studying the clinical and laboratory aspects of the GH axis and in caring for patients with disturbances of this axis. Dr. Slyper also qualifies as a significant contributor, as is evident from his thoughtful and cautious approach to the use of GH as a pharmacologic agent. He published his thoughts initially in 1995 in *Medical Hypothesis* in an article entitled "How Safe and Effective Is hGH at Pharmacologic Dosing?" He has updated his presentation for publication in this issue of *GGH*.

Other recent important articles concerning the use of human GH (hGH) that our readers may wish to consult include (1) an article from the Committee on Drugs and the Committee on Bioethics of the American Academy of Pediatrics, published in *Pediatrics* (1997;99:122-129), that is entitled "Considerations Related to the Use of rhGH in Children"; (2) an article edited by Cuttler et al, entitled "Short Stature and GH Therapy: A National Study of Physician Recommendation Patterns," published in *JAMA* (1996;276:531-537); and (3) an article from a committee appointed from the Lawson Wilkins Pediatric Endocrine Society, entitled "Guidelines for the Use of GH in Children With Short Stature," published in *J Pediatr* (1995;127:857-867). The first two are abstracted in this issue of *GGH*; however, reading the articles in their entirety is highly recommended.

Letters to the Editors expressing the thoughts and opinions of our readers regarding the lead articles of this issue of *GGH* and related topics are both welcome and encouraged. These can be sent c/o Robert M. Blizzard, MD, 1224 West Main Street, Suite 701, Charlottesville, VA 22903 or faxed to 804-977-9450.

For the Editorial Board,
Robert M. Blizzard, MD
Editor-in-Chief

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rhGH administration, new safety issues have been raised, such as tumor recurrence and leukemia. Finally, new therapeutic options may become available—for example, GH-releasing hormone (GHRH), GH-releasing peptide (GHRP), and IGF-1—and the use of GH must be evaluated in light of such proposed alternative treatments.

APPROVED INDICATIONS FOR GH THERAPY IN CHILDHOOD

As stated above, there are 3 FDA-approved indications for the use of GH therapy to promote growth during childhood: childhood GHD, growth failure associated with CRI, and short stature associated with TS. Each of these diagnostic categories is worthy of comment.

Childhood Growth Hormone Deficiency

On the surface, this would appear to be the least controversial indication for GH treatment. Children with classic GHD have severe growth failure, and GH ***replacement*** should lead to catch-up growth and the potential for achieving normal adult height. The problem here lies not in the therapy but in the diagnosis.[1] The potential pitfalls in provocative GH testing have been described in detail[2] and are presented in Table 1. These pitfalls have made the diagnosis of childhood GHD less clear-cut.

While measurement of serum concentrations of GH-dependent peptides such as IGF-1, IGFBP-3, and ALS has certain advantages, currently it is difficult to determine whether testing these factors is superior to provocative GH testing in identifying partial GHD and in predicting the clinical response to GH treatment.[3] Biochemical diagnostic strategies are further strained by the testing of children who do not have clear evidence of growth failure. Accordingly, it is proposed that auxologic criteria be employed as working guidelines when considering a diagnosis of GHD (Table 2).

Thus, the diagnosis of GHD should integrate auxologic and biochemical strategies, which need not be limited to provocative GH testing but can include other aspects of the GH/IGF axis, such as GH-dependent IGF-1, IGFBP-3, and ALS. Even an integrated diagnosis, however, should be considered provisional, especially for partial GHD, and the diagnosis must be reconsidered in the child who does not respond appropriately to conventional GH therapy. It is worth pointing out that even though GH has been used in the treatment of GHD for 40 years, there still has never been a ***controlled*** trial demonstrating the impact of therapy on adult height.

Even when the appropriate diagnosis of GHD has been established, controversies remain, such as what the appropriate starting dose of GH should be. While studies do demonstrate a dose-response for GH,[4] the slope of this correlation is relatively shallow, with only modest increases in growth rate seen when the GH dosage is increased, for example, from 0.025 to 0.05 mg/kg/d or from 0.05 to 0.1 mg/kg/d. On the other hand, it has been argued that there are psychosocial benefits to returning a short child to the normal growth curve as rapidly as possible, and that treatment at the larger dose best assures the eventual attainment of normal adult height.[5] Given the significant cost of GH and the potential for dose-related side effects,[6] it seems best to individualize the therapeutic approach. For example, in children who are diagnosed early in life or when short stature is mild, beginning treatment with a dosage of 0.025 mg/kg/d and reserving the option of increasing the dose if at any time the growth response attenuates is most logical. The young child with "true" or severe GHD should respond well initially to the lower dosage (0.025 mg/kg/d). However, for the older child with GHD or the younger GHD child with severe short stature, a higher initial dosage of GH (0.05 mg/kg/d) is appropriate. It is important to recognize, however, that high doses of GH may carry an increased risk of side effects or of accelerated entry into puberty. In general, it is always best to individualize the therapeutic approach, with careful assessments of clinical responsiveness and potential adverse effects.

Table 1
Potential Pitfalls in Provocative GH Testing

- The nonphysiologic nature of pharmacologic testing
- Difficulty in resolving conflicting data from 2 or more tests
- The inconsistencies in reproducing the response to the same pharmacologic tests
- The arbitrary definition of a "normal" response
- Age variability and sex steroid effect of the GH response
- Interassay variations among various GH radioimmunoassays
- The impact of nutrition, adiposity, and emotional state on the GH response
- Cost of tests
- Risks of testing, eg, hypoglycemia induction

Growth Failure Associated With Chronic Renal Insufficiency

In the United States, growth failure associated with CRI was the second FDA-approved indication for GH. This approval was based entirely upon short-term data. While a control group was employed, neither long-term studies nor controlled investigations carried out until attainment of adult height have been performed to date.[7] The physiologic basis for growth failure secondary to CRI remains unknown, and consequently, the rationale for GH therapy must be considered pharmacologic rather than physiologic. Although several studies have demonstrated increased serum concentrations of IGF-inhibitory binding proteins, it is not clear that GH works in these patients by normalizing IGFBP levels or increasing free IGF-1.[8] Long-term safety issues surrounding the use of GH in children with CRI and/or following kidney transplantation still need to be addressed.

Short Stature Associated With Turner Syndrome

There is little evidence to support the concept of an endocrine etiology for the characteristic growth failure of patients with TS. Serum concentrations of GH and IGF are normal for age in prepubertal girls with TS, despite the fact that growth failure is typically demonstrable by midchildhood or earlier.[9] Considering TS as a form of skeletal dysplasia is probably appropriate, and, accordingly, GH therapy should be recognized as being pharmacologic rather than physiologic. A large number of studies have demonstrated growth acceleration with GH treatment in TS, although the growth response does not match that observed in naive GHD children.[10] Furthermore, several studies have shown a positive effect of GH on adult height.[11-13] In the Genentech-sponsored clinical trials, GH-treated patients had an 8.4-cm increase over their baseline projected adult height; subjects receiving both GH and oxandrolone had a 10.3-cm increase.[13] While this study did not include a placebo control group followed to adult height, a matched historical control group had a mean adult height identical to that of the treated patients' baseline projected adult height. This salutary effect of GH on adult height has been demonstrated in several other studies,[11,12] although another reported a more modest improvement in adult height.[14]

However, most subjects in the latter studies[11-13] generally have been characterized by a relatively advanced chronologic and bone age and/or a relatively early introduction of estrogen for feminization. Given the effect of estrogen on epiphyseal fusion, estrogen therapy unequivocally will compromise the net growth response to GH. From the aspect of maximizing the adult height of each TS patient, the critical issue appears to be the ***number of estrogen-free years that GH is administered.*** Ideally, therapy should be individualized for each patient in an effort to both normalize growth and adult height and allow pubertal development at a relatively normal age. This approach requires early diagnosis of TS and initiation of GH treatment by midchildhood or earlier, before the patient's height falls below the 10th percentile on the normal female growth chart.

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Table 2
Auxologic Guidelines for the Diagnosis of GH Deficiency

- Severe growth retardation (height >3 standard deviations [SD] below the mean for chronologic age) in the absence of an alternative explanation
- Moderate growth retardation (height -2 to -3 SD below the mean for age) plus growth deceleration (height velocity <25th percentile for age) in the absence of an alternative explanation
- Severe growth deceleration (height velocity <5th percentile for age) in the absence of an alternative explanation
- A predisposing condition, eg, cranial irradiation, plus growth deceleration
- Other evidence of pituitary dysfunction, eg, other pituitary deficiencies, neonatal hypoglycemia, microphallus

Transition From Childhood to Adult GHD

Treatment of adult GHD is now an FDA-approved indication for GH. While the efficacy of GH in correcting the metabolic consequences of and improving quality-of-life issues associated with long-standing adult GHD has been demonstrated, the benefit and/or effect of continuing GH treatment in childhood-onset GHD following epiphyseal fusion have not been demonstrated. Although it may appear logical to continue therapy without a hiatus, the benefits, if any, of this approach require analysis through randomized, controlled trials. Unfortunately, significant compliance issues are inevitable in an adolescent population to whom no immediately obvious benefit of continued parenteral medication is apparent. Additionally, continuing GH treatment in adult patients with childhood-onset GHD requires standardization of the retesting of these patients, since as many as 60% to 70% of them will have normal provocative GH results when reevaluated as adults.[15]

THE USE OF GH FOR UNAPPROVED INDICATIONS

GH has been used for treatment of short stature associated with skeletal dysplasias, dysmorphic syndromes, metabolic conditions such as hypophosphatemic rickets, idiopathic intrauterine growth retardation (IUGR), and idiopathic short stature (ISS). In the absence of demonstrable GHD, the growth response to GH treatment is generally modest. None of these conditions has been studied with sufficient numbers of subjects to allow adequate evaluation of the impact of therapy on adult height; in particular, no concurrent control group has been employed in long-term investigations. This is of particular importance in those conditions for which there are insufficient historical data to allow comparison of the observed growth response with the natural history of the disorder and in those disorders that are by nature heterogeneous, as in ISS.

The issue of GH treatment for ISS is particularly complex.[16-19] Undoubtedly, some of these patients

have constitutional delay of growth and maturation and can be expected to attain normal adult heights, even in the absence of therapy. Furthermore, the limitations in our ability to accurately diagnose GHD in children, as discussed above, may make it difficult to distinguish between some patients with ISS and others with partial GHD. There are some children who have been diagnosed as GHD who are in fact endocrinologically normal, just as there are some children categorized as having ISS who have either partial GHD or IGF deficiency. An additional concern with GH treatment of ISS is the issue of whether GH therapy results in an earlier onset of puberty than might have otherwise occurred, resulting in early epiphyseal fusion and the forfeiture of whatever height gain might have been attained during the early years of GH treatment.[20] These issues make it difficult to strongly recommend GH treatment for patients in these categories. On the other hand, there are undoubtedly ISS or IUGR patients who respond effectively to GH treatment, sometimes in as robust a manner as GHD patients. Accordingly, it is recommended that such patients be treated as part of prospective clinical trials or on a case-by-case basis, following a full discussion of the potential benefits and risks of therapy.

THE FUTURE OF GH THERAPY

It is anticipated that GH will remain the treatment of choice for children with classic GHD, at least for the near future. Improved formulations, easier methods of reformulation and routes of administration, and long-lasting GH preparations should all enhance compliance with and, ultimately, the success of GH treatment. As more data are accumulated on the cost:benefit ratio of higher GH doses and as more experience is gathered on the adverse effects of high GH doses, a better rationale for dosing should be developed. Therapy for non-GHD short children, such as those with CRI or TS, will continue to entail pharmacologic doses of GH to obtain short-term increases in height. Ultimate adult heights will be increased in TS patients, but whether the same will apply to patients with CRI or those with ISS and other causes of short stature remains to be determined. It is incumbent upon the endocrine community, pediatricians, and internists to continue careful monitoring of GH recipients for both short-term and long-term side effects. As experience accumulates with GHRH and other GH secretagogues, such as the GHRPs, we will be able to determine whether such therapeutic options provide any benefits over GH, even if only to a subset of patients.

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How Safe and Effective Is Human Growth Hormone at Pharmacologic Dosing?

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Until 1985, cadaveric growth hormone (GH) was the sole source of human GH (hGH). The recommended dosage of between 0.24 and 0.3 IU/kg/wk or approximately 0.1 mg/kg/wk represented a compromise between the limited hormone supply and obtaining an optimal growth response. Since the introduction of biosynthetic hGH, the recommended dosage has increased to 0.3 mg/kg/wk (0.78 to 0.9 IU/kg/wk). This is roughly triple that used in the past and about 3 times the rate of endogenous GH production, except at adolescence.[1] Use of this dose has led to growth acceleration in conditions in which GH may be partially deficient, namely idiopathic short stature,[2] as well as conditions in which GH secretion is normal, such as Turner syndrome,[3] bone dystrophies,[4] and intrauterine growth retardation.[5] Irrespective of their endogenous GH status, many short children will experience growth acceleration on currently recommended hGH doses. It is not surprising, therefore, that conditions other than classic (severe) GH deficiency (GHD) now account for more than 40% of patients treated in this country with biosynthetic hGH.

It is clear from these studies, however, that currently recommended doses of hGH often have gone beyond providing physiologic replacement and are now phar-

macologic. In Turner and other syndromes, for example, GH treatment is clearly aimed at producing a supraphysiologic effect, namely, a final height beyond that dictated by genetic potential.[3] This is a unique venture. Never before in the history of medicine has a biologic agent been used in an attempt to produce such widespread and permanent physical changes. However, to anticipate that this can be achieved without undesirable side effects also would be unprecedented. With this in mind, issues of safety should be of paramount concern. Unfortunately, there is a limit to which the available data from long-term replacement dosing or short-term pharmacologic dosing can be used to guarantee the ultimate safety of pharmacologic GH therapy.

There is a general consensus that over the short term pharmacologic GH dosing is reasonably safe.[6] An increased risk of intracranial hypertension and slipped capital femoral epiphysis has been documented, but the incidence of these complications is not large.[6] Of all potential complications, however, it is the risk of malignancy that should be of greatest concern. To date, 44 patients have developed leukemia following GH treatment, 12 of them from Japan.[6] The malignancies reported have been stem cell malignancies, such as leukemias, lymphomas, or thymomas.[7] Many of the patients had received pituitary-derived GH at moderate doses, and some had been off therapy for several years at the time of diagnosis. Of the 12 cases from Japan, 8 had idiopathic GHD and none of the usual risk factors for leukemia such as chemotherapy, radiation therapy, or preexisting malignancy. The data from Japan, therefore, remain unexplained.[8] It is generally agreed that there is insufficient evidence to incriminate GH therapy as a cause of leukemia, leukemic relapse, or tumor recurrence.[6] Nevertheless, a high index of suspicion needs to be maintained, since GH has the potential for being carcinogenic.[7] Acromegalic patients are at increased risk for developing benign and malignant tumors, particularly colon polyps and adenocarcinoma.[9] In a small group of acromegalic patients with active disease, 53% had colonic polyps.[10] In rats, both hypophysectomy and large doses of GH influence the effect of carcinogens.[7] Intraperitoneal injection of large doses of purified pituitary-derived GH into rats for up to 485 days resulted in rapid growth as well as neoplasia in multiple organs: lymphosarcomas of the lung, adrenocortical and adrenomedullary carcinomas, solid ovarian tumors, and breast tumors.[7] Such toxicology studies may have little relevance when considering physiologic replacement dosing, but the situation may be otherwise for pharmacologic dosing.

Of possible relevance to this issue are observations that melanocytic nevi of children with hypopituitarism and Turner syndrome show increased growth, increased proliferative activity, and atypical signs of differentiation during GH therapy, although there is no evidence of neoplasia.[11,12] Increased chromosome fragility also has been demonstrated in lymphocytes obtained 3 to 12 months into the treatment of normal short children, in addition to an increase in spontaneous chromosome rearrangements and a significant increase in bleomycin-induced aberrations.[13]

Hyperinsulinemia and insulin resistance have been noted in Turner syndrome and normal short children during GH therapy.[14,15] The absence of diabetes in all but a few patients in no way excludes the possibility of sequelae from a childhood spent in a state of hyperinsulinemia and insulin resistance.[7,14] Doubtless, most children will suffer no long-term effects, but this may not be the case for patients who already have a predisposition to atherosclerosis, diabetes, or hypertension. We have to admit that our knowledge of the natural history of these diseases is limited, and it may be decades before we can say with certainty that treatment has no influence on the development of these conditions.

Between 53% and 76% of patients with acromegaly develop joint problems, with a delay of approximately 10 years between the onset of acromegaly and the appearance of arthropathy.[16] Typical joint changes include widening of the joint spaces, osteophyte formation, joint capsule calcification, and mineralization of ligamentous insertions.[17] These changes are irreversible. Whether joint disorganization also occurs in developing joints as a consequence of higher-dose GH therapy will not be known for years. Reports of

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

avascular necrosis of the femoral head and slipped capital femoral epiphyses in a few treated children may or may not be the tip of the iceberg.[18] The potential for joint disturbances following high-dose GH treatment could be a particular concern in conditions with preexisting abnormalities of the growth cartilage. Modest short-term growth acceleration has been achieved in some patients with achondroplasia using pharmacologic GH dosing.[4] The basic defect in this condition is in the fibroblast growth factor receptor 3 gene and relates in some manner to abnormal cartilage growth and endochondral ossification. A bone dysplasia also contributes to the short stature of Turner syndrome, and an abnormality of cartilage cannot be excluded.[19,20] GH-treated children with chronic renal failure could be another group at high risk for bone and joint complications.[18] The extent to which abnormal joints and bones can be increased beyond their genetic potential and yet retain complete functional integrity throughout adulthood is not known.

Even if we follow a safety-first approach to pharmacologic GH treatment, the benefits of therapy are another important issue. We should not be placing any child at *unnecessary* risk. While higher-dose GH has improved the height prognosis for children with classic (severe) GHD, the situation is far more ambiguous with respect to children with other forms of GH insufficiency. This is a relevant concern, since these children now constitute a large proportion of children being treated with GH.

Neurosecretory GH dysfunction was first observed in children who had undergone prophylactic cranial irradiation for leukemia. Frequent sampling of endogenous GH over 24 hours demonstrated diminished GH secretion; GH pulses were attenuated in size and diminished in number.[21] Similar secretory patterns were subsequently found in other short, poorly growing children who had not undergone cranial irradiation and who had "passed" provocative GH stimulation testing.[22] The concept arose of a spectrum of GH insufficiency, ranging from short but normally growing children at one end of the spectrum, and children with classic GHD at the other, and a group of children with borderline to subnormal growth and partial GHD in between. At the same time there was a loosening of the "pass-fail" criteria for GH stimulation testing and a cutoff of 10 ng/mL rather than 5 to 7 ng/mL was adopted.[23]

Despite wide acceptance of neurosecretory GH dysfunction as a distinct clinical entity, there is much about this syndrome that is extremely ambiguous. There are, for example, no objective criteria for its diagnosis. Twenty-four-hour GH monitoring is expensive and labor-intensive, and has remained primarily a research tool. It also seems to be no better at diagnosing GHD than stimulated GH levels.[24] The diagnostic cutoff levels used during GH stimulation testing are recognized as arbitrary.[25] The finding of substantial discrepancies between one GH assay and the next, to the point that the diagnosis of GHD may depend on which assay is used, has highlighted the inadequacies of provocative testing.[26]

By default, therefore, a subnormal growth velocity often becomes the decisive factor in the decision to initiate GH treatment. However, the measurement of short-term growth velocity is itself subject to biases and inaccuracies. Growth velocities in the autumn and winter may be more than 2 cm/y lower than during the rest of the year, and a growth velocity of less than 2.5 cm/y during these seasons may be normal.[27] One study found that growth velocity was significantly higher after GH testing than before testing (3.4 cm/y versus 5.1 cm/y for prepubertal children and 3.4 cm/y versus 6.3 cm/y for pubertal children). An explanation for this odd finding may be that growth velocities prior to testing were transiently low, leading to a selection bias in referral.[28] The 95% confidence limits of a single height measurement performed by skilled personnel is ±0.5 cm.[29] There is a similar lack of precision for measuring yearly growth velocities. For a short normal child growing along the 25th percentile, the confidence limits for yearly growth velocity span the 8th to 52nd percentiles. In general, the lower limit would be considered abnormal while the upper limit would be within the normal range. For measurements taken by inexperienced personnel or at less than 12 months apart, confidence limits would be even greater. Over 2 years, there is no correlation between year to year growth velocities, suggesting that short-term growth velocity is an unreliable means of predicting future growth.[29] The ambiguities surrounding the diagnosis of neurosecretory dysfunction no doubt account for some of the discrepancies in GH prescribing practices between one pediatric endocrinologist and another.

Recent interest in treating GHD adults has focused attention on the question, "What percentage of patients diagnosed with GHD in childhood truly have this condition?" The answer should give pause for thought. Tauber et al[30] found that 71% of 98 adults previously diagnosed as having partial GHD (peak GH response between 5 to 10 ng/mL) and 36% of 33 adults diagnosed with complete GHD (peak GH response <5 ng/mL) had normal stimulated GH peaks of greater than 10 ng/mL on a single stimulation test.

Not only is the diagnosis of partial GHD ambiguous, but the results of GH treatment also are unclear. For any child receiving GH, puberty appears to be an important dividing line in terms of therapeutic response. Testosterone and estrogen increase the amplitude of GH pulses, and a pubertal increase in GH accounts in part for the growth spurt of puberty.[31] This is, however, a 2-way relationship, as GH also influences pubertal

In Future Issues

Molecular Physiology of Leptin and Its Receptor
Yiying Zhong, PhD, and Ron L. Leibel, MD

Growth Hormone Replacement In Adult GHD Patients
Peter Sonksen, MD

Insulin, IGF-1 and IDDM: Recently Implicated Genetic Loci
Cheryl L. Deal, PhD, and Constantin Polychronakos, MD

sex steroid secretion.[32] GH treatment of GHD and non-GHD children to final height results in accelerated pubertal progression and a pubertal decrease in height standard deviation scores (SDS) for bone.[33-36] The acceleration in pubertal progression is dose-dependent. In a large group of male children with isolated GHD, a doubling of GH dose from 15 IU or 5 mg/m^2/wk to 30 IU or 10 mg/m^2/wk increased the rate of pubertal maturation but had no effect on growth velocity.[37] An earlier onset of puberty also was noted in a controlled study of non-GHD children, and this study concluded that therapy may actually have led to a decrease in final height.[34]

The implication of these observations appears to be that for children who are not truly GHD, the closer to puberty that GH treatment is initiated the less likelihood of a gain in final height. If treatment is started early in childhood, there is a greater chance of exceeding genetic potential. However, to accomplish this, supraphysiologic doses of GH need to be administered throughout childhood, resulting in a greater potential for long-term complications.

Recommendations

Based on this discussion, I propose the following recommendations, appreciating that many of these points are out of line with the current practices of many pediatric endocrinologists:

1. The recommended dosage of GH treatment of 0.3 mg/kg/wk is a high one for the initial treatment of children with severe (classic) GHD. Treatment should be started at a lower dose and further dose changes titrated against the observed growth effect.

2. Families of short children who pass provocative testing and in whom pharmacologic GH treatment is contemplated should be informed that negative short-term data provide no assurance as to the ultimate safety of pharmacologic GH therapy and that the benefits of treatment in terms of final height are unknown. Families of children with Turner syndrome who are about to be placed on the newly recommended GH dosage of up to 0.375 mg/kg/wk also should be informed that there is little information on the short-term safety of this dose and none on its long-term safety.

3. For poorly growing peripubertal children, GH testing should be accompanied by sex steroid priming so as to exclude the transient, physiologic GHD present in many youngsters with constitutional delay of puberty. Sex steroid priming has gone out of favor in recent years, but could be used far more extensively.

4. A multicenter *controlled* trial should be organized to follow to final height children specifically with neurosecretory GH dysfunction or partial GHD treated with currently recommended doses of GH. It can no longer be taken for granted that these children benefit from therapy. Noncontrolled studies using estimated heights or historical controls are incapable of demonstrating conclusively the benefits of treatment. In my opinion, a study of this nature should take priority over other contemplated growth studies investigating new indications for GH treatment with ever increasing doses.

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Short Stature and Growth Hormone Therapy: A National Study of Physician Recommendation Patterns

The objective of this study was to learn the attitudes of pediatric endocrinologists (PEs) regarding prescribing growth hormone (GH) to short children. Of 534 anonymous surveys, 434 (81.3%) were returned. Extensive planning of the questionnaire permitted the collection and analysis of data revealing the attitudes of 340 of the 434 respondents who currently manage short stature in children. Of the children currently being treated, 58% were GH deficient (GHD) and 15% had Turner syndrome (TS). The remaining 27% had other causes for short stature. Eight case histories, differing only in physiologic growth variables (extent of short stature, growth velocity, normal or abnormal bone age) were presented and the respondents were asked whether they were likely to recommend GH for each case. Three additional sets of decisions focusing on the contingency variables of price and family wishes also were included in the questionnaire. The first 2 contingencies proposed that the price of GH therapy fell from approximately $13,000 per year to $2,000 per year or $100 per year. In the third contingency, physicians were asked their recommendation if the family strongly desired GH therapy, assuming that the price remained at current levels.

Analyses of the data revealed 3 noteworthy patterns in the responses. First, 68.1% agreed that GH use for non-GHD short stature has increased in the past 5 years and that the physician's knowledge about family finances is marginal in the overall decision-making process whether to prescribe GH. Second, PEs believe that short stature matters and has dysfunctional emotional impact on many children and adults. Third, a lack of consensus existed among the PEs regarding the perceived efficacy (adult height and long-term adverse effects) of GH therapy for non-GHD children.

In applying a logistic model to physicians' decisions to recommend GH, 3 sets of predictors were used: *(a)* the physiologic growth variables previously discussed; *(b)* contingency variables, ie, treatment cost and family wishes; and *(c)* physicians' beliefs about short stature and GH treatment. The growth rate was very important, as the likelihood of GH being prescribed increased 3.4-fold for a growth rate below the 3rd percentile versus the 3rd to 10th percentiles. A height falling below -3 standard deviations (SD) increased by 2.8-fold the likelihood of GH being prescribed than if the patient was between -2 and -3 SD below the mean. A normal bone age instead of a delayed bone age increased the recommendation to use GH. Also, boys with comparable shortness to girls, corrected for sex, were 1.3-fold more likely to receive GH than girls. Physicians were sensitive to cost and would have significantly increased recommending the use of GH if it cost $2,000 per year instead of $13,000 per year. A cost of $100 per year would have further significantly increased recommendations. Family wishes clearly influenced the recommendations made by many physicians. In addition, the odds of a positive recommendation increased 13% if the physician believed GH would add at least 1 inch to the ultimate height of non-GHD children. The authors concluded from analyses of the data that physiologic factors, contingency factors, and belief factors exert independent and additive effects on recommendations for GH therapy.

The conclusions that can be drawn from this study have several implications for GH and analogous interventions such as treatment of attention deficit disorder, in vitro fertilization, and genetic testing. Like GH therapy, these analogous interventions hold promise for increasing the quality of life for a targeted patient population, but with uncertain risks and benefits and often at considerable financial cost. As HMOs try to limit the use of GH because of a lack of consensus concerning its use, even referrals for short stature may be limited, which would be unfortunate since referral not only should address whether GH is recommended but also elucidate whether a cause for short stature requiring therapies other than GH is present.

Cuttler L, et al. *JAMA* 1996;276:531-537.

Editor's comment: *This article demonstrates the optimal use of scientific methodology in constructing a questionnaire that will yield interpretable data. Our readers are encouraged to review the entire article for its multiple contributions, particularly the insight it yields into the factors that may prompt the prescribing of other agents that may improve the patient's quality of life but not necessarily significantly improve the ultimate height of the patient.*

Although originally published in 1996, this article seemed worthy of abstracting in GGH *as it is an excellent corollary to the 2 lead articles in this issue.*

Robert M. Blizzard, MD

Considerations Related to the Use of Recombinant Human Growth Hormone in Children

This is an excellent overview of different aspects of the use of growth hormone (GH) by the Committee on Drugs and the Committee on Bioethics of the American Academy of Pediatrics. Some important points are summarized here, but the reader is encouraged to review the complete article published in *Pediatrics* 1997;99:122-129.

BACKGROUND

Recombinant GH Products

The biosynthetic process involves a chemical synthesis of the DNA fragment encoding the first 24 amino acids and complementary DNA copies of messenger RNA prepared from human pituitary cells. The entire DNA sequence is intro-

duced into a bacterium, *Escherichia coli*, which enables the synthesis of GH. The products currently available in the United States differ in that somatrem (Protropin®, Genentech, Inc.) contains an additional methionine group whereas somatropin (Nutropin® and Nutropin AQ®, Genentech, Inc., and Humatrope®, Eli Lilly & Co.) are identical to human GH.

Optimal dosing strategies have not been developed fully for any product. Most pediatric endocrinologists recommend 0.18 to 0.30 mg/kg/wk depending on the product, given in equally divided daily doses 6 or 7 times a week.

Problems in the Diagnosis of Growth Hormone Deficiency

Because random fasting serum GH levels do not differ between GH-deficient (GHD) and non-GHD patients, other physiologic and pharmacologic tests have been developed to identify GHD patients. Classic severe GHD can be diagnosed if the peak stimulated value on 2 tests is ≤ 10 µ/L or less in association with delayed bone age and slow growth rate. Other forms of GHD, ie, partial GHD, cannot be fully diagnosed by these pharmacologic tests. Thus, GH treatment should be considered primarily on clinical grounds for those patients who present with slow growth velocity and delayed bone age.

Goals for rhGH Administration to Children With Short Stature

Much of the controversy surrounding the use of GH is related to the absence of well-defined goals for therapy. Most physicians agree that GH therapy should be reserved for patients with either classic GHD or some other conditions that exhibit a demonstrable benefit with GH treatment, such as Turner syndrome (TS) or chronic renal insufficiency (CRI). The goal of GH treatment is to attempt to maintain age-appropriate growth and to attain a final adult height that is consistent with the patient's genetic potential. In contrast, other physicians argue that all patients with short stature (SS) may be given a trial because of the physical and psychosocial handicaps associated with this condition. However, there is no universal consensus regarding the definition of SS. Some physicians define SS as a height below a certain percentile on a standard longitudinal height chart; others believe that height velocity for age and sex is the more appropriate indicator.

Unfortunately, there is an absence of generally accepted criteria for diagnosing "inadequate secretion," partial GHD, or GH dysregulation. Also, some children with various forms of SS respond to GH therapy with accelerated growth velocity. For these reasons, some pediatric endocrinologists believe that GH should be made available for a therapeutic trial to all children with SS regardless of its etiology as long as they have a decreased growth rate.

A survey conducted by the American Academy of Pediatrics revealed that abnormal GH levels during provocative testing followed by decreased growth velocity in SS patients were the criteria used most frequently by pediatric endocrinologists.

Risks Associated With GH Therapy

Short-term treatment with GH has been associated with few side effects, but long-term risks are still unknown. The most frequent side effects are as follows:

- antibody formation at an overall level of 10% or lower with no clinical effects.
- pseudotumor cerebri related to the use of GH and/or insulin-like growth factor 1. Benign intracranial hypertension with papilledema has been reported rarely. Cessation of GH therapy has reversed the symptoms in reported cases. In some cases, spontaneous resolution has occurred in spite of continued treatment.
- unusually lean and inappropriately muscular appearance due to increased cellular metabolism.
- potential physiologic and psychologic trauma related to years of regular injections.
- theoretical concerns that GH therapy might be related to an increased risk of malignancy. Although an increased incidence of leukemia among patients treated with GH has been reported in Japan, a recent US/Canadian survey did not show an increased risk for leukemia or brain tumor unless other risk factors were present, ie, previous radiation therapy or chemotherapy.

ETHICAL ISSUES

Since there are no data demonstrating improvement in ultimate height in patients with nonclassic GH, one question should be addressed: Is GH therapy acceptable for children who do not fulfill the criteria for classic GHD?

It also is well known that being tall is indisputably viewed as a benefit in our culture, and is associated with multiple advantages, including higher income, academic achievement, self-esteem, and social status. The concepts of normality and abnormality are difficult to define, and they incorporate many sociocultural variables. In most cases, a better alternative may be to help children and their families to achieve pride and fulfillment on their own terms.

There also are important economic issues regarding GH. Some children who do not have classic GHD now receive GH therapy, and these children are likely to come from the more financially well-off sector of society. On the other hand, many children lack access to the most basic health care, and it would be ethically inappropriate to spend scarce monetary resources to provide GH therapy to anyone who is not classically GHD or truly GH-resistant.

CONCLUSIONS AND RECOMMENDATIONS

1. Therapy with GH is medically and ethically acceptable for:
   - children with classic GHD
   - children with CRI who are awaiting kidney transplantation
   - girls with TS
   - children whose extreme SS keeps them from participating in basic activities of daily living and who have a condition for which the efficacy of GH therapy has been demonstrated.
2. Two key considerations argue against widespread administration of GH therapy to other short children:
   - There could be unknown long-term risks.
   - The treatment could result in either no increase or only an insignificant increase in final adult height.

3. Therapy may be justified for children whose height could prevent them from participating in the basic activities of daily living.
4. Pediatricians should be alert to commercial efforts to stimulate parental interest in GH therapy as an avenue for improving athletic ability and other forms of social "success" for their children.

Fima Lifshitz, MD

Increased Height in Patients With Medulloblastoma

Robertson et al report their surprising findings from chart reviews of 85 patients with medulloblastomas seen at the University of Iowa College of Medicine from 1963 to 1995. These patients (64 children and 21 adults) had their height and weight documented on standardized growth charts before treatment of their tumors. The data show that 22.4% of these patients were above the 95% curve (see Table) in height. In a comparison group of patients with cerebellar astrocytomas, only 7.1% were above the 95% curve for height. Thus, there is a clear difference between linear growth in the 2 different groups. Most of the increased height was in male patients; however, 56 of the 85 patients were male. Interestingly, patients who presented as adults also were taller than expected at diagnosis.

The authors related that medulloblastoma cell lines can express different levels of growth factors, including epidermal growth factor, platelet-derived growth factor, transforming growth factor, and insulin-like growth factor (IGF). They note that since the adults in the study also were above normal height, something must have occurred that predated the development of neoplastic cells.

Robertson SC, et al. *Neurosurgery* 1997;41:561-566.

Editor's comment: *These authors have presented some very interesting and intriguing data. Since the study was retrospective, there are no carefully collected hormonal data from the individuals, ie, testosterone, growth hormone, IGF-1, etc. Nor do the authors present any data regarding the pubertal status of the children at the time of diagnosis. It is conceivable that some of the children with medulloblastoma may have had early puberty, which would account for their being taller than expected. Postoperatively, growth failure is the rule rather than the exception in these individuals. Although we have no data on final heights in the children, one would anticipate that those patients diagnosed and treated as children would not end up being tall adults. The data do suggest, however, that it is important for pediatric endocrinologists to continue to encourage their neurosurgical colleagues to evaluate the hormonal status of their patients preoperatively as well as after treatment.*

William L. Clarke, MD

Preoperative Height and Weight of Patients With Medulloblastomas[a]

Medulloblastoma Preoperative Curves (%)	Height		All Patients	Weight		
	M	F	Total (%)	M	F	Total (%)
>95	14	5	19 (22.4)	6	0	6(7.1)
>90	18	8	26 (30.6)	9	1	10(11.8)
>75	36	11	47 (55.3)	15	2	17(20.0)
>50	46	18	68 (80.0)	32	10	42(49.4)
>25	55	25	80 (94.1)	44	14	58(68.2)
>10	55	29	84 (98.8)	50	18	68(80.0)
>5	55	29	84 (98.8)	54	23	77(90.6)
>0	56	29	85 (100)	56	29	85(100)

[a]Numbers in each column represent the total number of patients above or equal to the percentile group listed.
From Robertson SC, et al. Increased height in patients with medulloblastomas. *Neurosurgery*. 1997;41:561–566.

Prenatal Diagnosis From Fetal Cells in Maternal Circulation

Detection of fetal aneuploidy by noninvasive means has been a long-term goal of the prenatal diagnostician. Screening procedures based on measuring substances in maternal serum, for example, maternal serum α-fetoprotein, detect many instances of aneuploidy. However, many are missed, and this deficit has prompted the search for other strategies, including analyzing fetal cells circulating in maternal serum. Indeed, it has been known for many

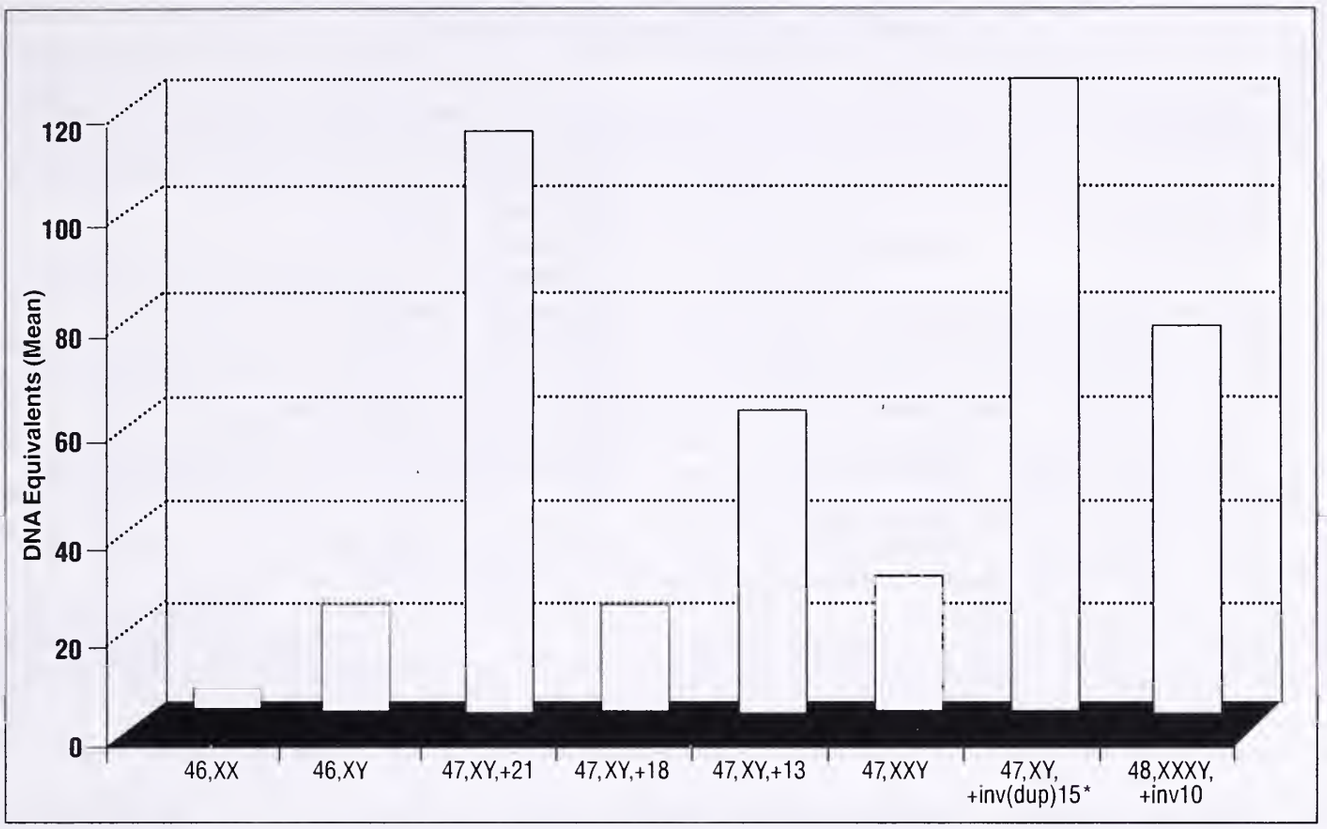

Figure. Bar graph demonstrating mean number of male fetal-cell DNA equivalents detected in maternal samples, stratified by fetal karyotype. Note that the highest number of male fetal-cell DNA equivalents is detected when the fetus has 47,XY,+21 or 47,XY,+inv(dup)15. The asterisk (*) indicates that the values for 47,XY,+inv(dup)15 are off the scale, with a mean value of 230.

From Bianchi DW, et al. PCR quantitation of fetal cells in maternal blood in normal and aneuploid pregnancies. *Am J Hum Genet* 1997;61:822-829. Published by University of Chicago.

years that a limited number of fetal trophoblasts, lymphocytes, granulocytes, nucleated erythrocytes, and platelets reach the maternal circulation. Several studies have explored various aspects of this issue, but differences in patient populations, cells studied, and methods used to enrich and analyze the cells have made it difficult to draw definite conclusions about the efficacy of this approach for prenatal diagnosis.

An article by Bianchi et al sheds light on the issue. In a large, multicenter clinical trial, PCR-amplified Y-chromosome sequences were obtained from 16-mL peripheral blood samples of 199 women carrying chromosomally normal fetuses and from 31 women with male aneuploid fetuses. They sought to determine the number of male cells, or their equivalent, that could be detected in maternal serum under different clinical conditions. Results were expressed as male fetal-cell DNA equivalents. There was no enrichment of cells so that values reflected the number of cells in the original sample and were not artifacts of enrichment; no distinction was made between the different cell types.

The mean number of male fetal-cell DNA equivalents from 90 women bearing 46,XY fetuses was 19, and more than 80% had values over 2 cell equivalents. There was no difference if the specimen was taken before or after amniocentesis. Surprisingly, Y-specific DNA sequences were found in about one fourth of women carrying female fetuses, although the values were lower than when male fetuses were being carried. Possible explanations for the presence of male cells in the maternal circulation were that the male cells had come from a male twin who had been lost early in the current pregnancy, or were from a previous transfusion from a male donor, or were from a previous pregnancy with a male fetus.

Most remarkable, Bianchi et al found a substantial increase in the number of male fetal-cell DNA equivalents if the fetus was aneuploid. There was a 6-fold increase in fetal cells detected in the maternal circulation when the fetus had trisomy 21(see Figure). Lesser increases were observed for trisomy 13 and 18, but fewer cases were assessed. The authors pointed out that this finding is consistent with pathologic observations of placental abnormalities in trisomies.

The authors concluded that a small but consistent number of fetal cells are normally transfused across the placenta into the maternal circulation. The number is increased substantially for aneuploid fetuses, especially for trisomy 21, which should make feasible detection of at least trisomy 21 from maternally circulating fetal cells.

Bianchi DW, et al. *Am J Hum Genet* 1997;61:822-829.
Goldberg JD. *Am J Hum Genet* 1997;61:806-809. Invited Editorial.

Editor's comment: *This technology has evolved from little more than wishful thinking 2 decades ago to an almost fea-*

sible prenatal diagnostic approach for trisomy 21 and potentially other aneuploidies. As pointed out in an accompanying editorial by Goldberg, there are problems that still must be resolved, such as the persistence of fetal cells from previous pregnancies. He notes 2 issues that must be addressed before widespread testing of fetal cells in maternal circulation is undertaken. The first issue is whether it should be offered to all pregnant women. The second issue is whether it should be used for gender selection.

William A. Horton, MD

Guest Editor's comment: *Advanced maternal age, variably defined as 35 to 40 years of age at the time of delivery, has long been a standard indication for prenatal diagnosis by chorionic villus sampling or amniocentesis. However, because the majority of infants with autosomal trisomies are born to women under 35 years of age, a number of approaches are used to screen for high-risk pregnancies among younger women. Clinical trials currently under way involving analysis of fetal cells in maternal circulation offer prospects for yet an additional approach.*

Maternal serum triple screening, ie, α-fetoprotein, HCG, and estriol, which assist in detecting neural tube defects, Down syndrome, and trisomy 18, provides a high detection rate for these entities. However, its use at 15 to 20 weeks of gestation, followed by the subsequent requirement for amniocentesis if the screening is suspicious for definitive diagnosis, is too late in pregnancy for use by many women. Transvaginal ultrasonography also is used to detect birth defects. Taiplae et al recently published their experience in detecting increased nuchal translucency in 10,000 unselected pregnancies, reporting a sensitivity of 54% for the detection of trisomy 21.

It is reasonable to assume that for the foreseeable future a combination of maternal serum triple screening, ultrasonography, and very possibly, analysis of fetal cells in maternal circulation will be used for testing pregnancies of younger women. However, none of these techniques is currently sufficiently sensitive or specific enough to obviate standard cytogenetic analysis of the fetus to arrive at a confident prenatal diagnosis of an autosomal aneuploidy.

Taiplae P, et al. *N Engl J Med* 1997;337:1654-1658.

Thaddeus E. Kelly, MD, PhD
Professor of Pediatrics and Genetics
University of Virginia School of Medicine

Gene Therapy: Promises, Problems, and Prospects

Gene therapy is a concept with which most of us are familiar. We know of its potential and that it has not lived up to this potential. However, few of us understand the biology that underlies gene therapy or appreciate the obstacles that gene therapists face. Fortunately, Verma and Somia have come to the rescue with a timely and concise review of the subject.

First, they point out that despite more than 200 clinical trials currently under way worldwide, there has been no clear success story yet. They consider the primary obstacles to be the lack of an efficient delivery system, the lack of sustained expression, and often a host immune response to therapy.

To Verma and Somia, the Achilles' heel of gene therapy is the delivery system. The properties of currently used gene therapy vectors, including retroviral, lentiviral, adenoviral, and adeno-associated viral vectors, are compared. Each has certain advantages, but each also has disadvantages. For example, retroviral vectors, which have been employed most widely in clinical trials, integrate well into host genomes and there are few immunologic problems; however, expression of the therapeutic gene is short lived. In contrast, adeno-associated viruses support long-term expression, but the logistics of producing large quantities of virus needed for therapy is difficult. As for adenoviral vectors, many patients have preexisting immunity to adenoviral proteins. Lentiviral vectors, which are related to HIV, show considerable promise. The authors conclude that the ideal vector will be constructed from elements of different viral vectors.

Regarding clinical trials, Verma and Somia note that more than half the trials initiated to date involve cancer; nearly 30 involve monogenetic disorders as listed in the Table (page 13). They also point out that most of the trials are Phase I (safety) studies, and that for the most part, no major toxicity problems have been encountered with the existing delivery systems.

Finally, the authors are optimistic about the future of gene therapy, basing their optimism on the steady progress being made in vector design.

Verma IM, Somia N. *Nature* 1997;389:239-242.

Editor's comment: *This is a short but informative review of the current status of gene therapy. It is written to be understood by the nongeneticist, yet provides a broad overview of the field.*

William A. Horton, MD

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Candidate Diseases for Gene Therapy

Disease	Defect	Incidence	Target Cells
Genetic			
Severe combined immunodeficiency (SCID/ADA)	Adenosine deaminase (ADA) in ~25% of SCID patients	Rare	Bone marrow cells or T cells
Hemophilia A	Factor VII deficiency	1:10,000 males	Liver, muscle, fibroblasts, or bone marrow cells
B	Factor IX deficiency	1:30,000 males	
Familial hypercholesterolemia	Deficiency of low-density lipoprotein (LDL) receptor	1:1 million	Liver
Cystic fibrosis	Faulty transport of salt in lung epithelium. Loss of *CFTR* gene	1:3,000 whites	Airways in the lungs
Hemoglobinopathies: thalassemias/sickle cell anemia	Structural defects in α- or β-globin gene	1:600 in certain ethnic groups	Bone marrow cells, giving rise to red blood cells
Gaucher disease	Defect in the enzyme glucocerebrosidase	1:450 in Ashkenazi Jews	Bone marrow cells, macrophages
α_1-Antitrypsin deficiency: inherited emphysema	Lack of α_1- antitrypsin	1:3,500	Lung or liver cells
Acquired			
Cancer	Many causes, including genetic and environmental	1 million/y in US	Variety of cancer cell types; liver, brain, pancreas, breast, kidney
Neurologic diseases	Parkinson's, Alzheimer's, spinal cord injury	1 million Parkinson's and 4 million Alzheimer's patients in US	Direct injection in the brain, neurons, glial cells, Schwann cells
Cardiovascular	Restenosis arteriosclerosis	13 million in US	Arteries, vascular endothelial cells
Infectious diseases	AIDS, hepatitis B	Increasing numbers	T cells, liver, macrophages

From Verma IM, Somia N. Gene therapy: promises, problems, and prospects. *Nature* 1997;389:240.

Changes in Bone Mineral Density, Body Composition, and Lipid Metabolism During Growth Hormone (GH) Treatment in Children With GH Deficiency

Adults with childhood-onset growth hormone deficiency (GHD) have reduced bone mass, increased fat mass, and disorders of lipid metabolism. The aim of the present study was to evaluate bone mineral density (BMD), bone metabolism, body composition, and lipid metabolism in GHD children before and during 2 to 3 years of GH treatment. The mean age of the 40 children participating in this study of bone metabolism and body composition was 7.9 years. An additional 17 GHD children participated in the study of lipid metabolism. Lumbar spine BMD, total body BMD, and body composition were all measured with dual energy X-ray absorptiometry. Volumetric BMD (or bone mineral apparent density [BMAD]) was calculated to correct for bone size. Standard deviation scores (SDS) were used to compare with normative data.

Lumbar spine BMD, total body BMD, and BMAD were all decreased at baseline. All these BMD variables increased significantly during treatment. The Table (page 14) presents the effects at various time points. Lean tissue mass SDS increased continuously. Fat mass SDS decreased markedly during the first 6 months and remained stable thereafter. The chemical parameters of bone formation and resorption at baseline did not differ from those of normals and then increased during the first 6 months of treatment. Serum 1,25 dihydroxyvitamin D increased continuously during treatment, whereas parathyroid hormone and serum calcium remained stable. The lipid profile was normal at baseline.

The authors conclude that children with GHD have decreased bone mass. BMD, together with height and lean tissue mass, increased during treatment, which also had a beneficial effect on lipid metabolism.

Boot A, et al. *J Clin Endocrinol Metab* 1997;82:2423-2428.

Editor's comment: *This interesting paper adds further data supporting the importance of GH treatment for GHD children, promoting linear growth and regulating different metabolic pathways. All patients presented in this study*

Mean of Different Variables at Baseline and During Growth Hormone Therapy

Variable	Baseline n = 38	At 6 Months GH Therapy n = 37	At 1 Year GH Therapy n = 33	At 2 Year GH Therapy n = 33
Lumbar spine BMD SDS	–1.62	–1.33[a]	–0.98[a]	–0.64[a]
Lumbar spine BMAD SDS	–0.51	–0.50	–0.37	–0.19[a]
Total body BMD SDS	–0.94	–1.35[b]	–1.02	–0.61[b]
Bone mineral content SDS	–2.29	–2.36	–1.52[a]	–1.24[a]
Lean tissue mass SDS	–2.72	–1.86[a]	–1.53[a]	–1.14[a]
Fat mass SDS	–0.02	–0.59[c]	–0.31[c]	–0.59
% Body fat SDS	0.93	–0.39[a]	–0.10[a]	–0.45[c]
Height SDS	–2.98	–2.32[a]	1.86[a]	–1.63[a]
Body mass index SDS	0.45	0.24	0.39	0.37

[a] $P < 0.001$; [b] $P < 0.02$; [c] $P < 0.01$ compared to baseline.

BMAD, bone mineral apparent density

BMD, bone mineral density

SDS, standard deviation score

From Boot A, et al. Changes in bone mineral density, body composition, and lipid metabolism during growth hormone (GH) treatment in children with GH deficiency. *J Clin Endocrinol Metab* 1997;82:2425. ©The Endocrine Society.

showed evidence of the anabolic effect of GH, as demonstrated by the increase in BMD, the increase in lean body mass, and the decrease in body weight. Some of these metabolic effects may be considered direct effects of GH replacement. An increase in the serum 1,25 dihydroxyvitamin D level has been reported during GH treatment due to renal inactivation induced by insulin-like growth factor 1, an indirect effect resulting in the beneficial increase in BMD.

The authors concluded that treatment had a beneficial effect on lipid metabolism. However, there were no significant changes found in lipid metabolism as baseline values were all normal. In my opinion, no conclusions can be drawn from the present study regarding the beneficial effects on lipid metabolism. Long-term studies in children need to be done since adults with GHD are at risk of hypercholesterolemia and coronary heart disease.

Fima Lifshitz, MD

Growth Hormone Therapy in Prepubertal Children With Noonan Syndrome: First Year Growth Response and Comparison With Turner Syndrome

The authors report that during the first year of administration of recombinant human growth hormone (rhGH; 0.15 U/kg/d given by daily injection) to 23 prepubertal subjects with Noonan syndrome (9.4 ± 3.0 years), the increase in height velocity was 8.5 cm, approximately twice the pretreatment growth rate. In a group of females with Turner syndrome of similar age at initiation of rhGH, the mean height increment was 8.1 cm during the first year of treatment. Four of 23 Noonan syndrome subjects had no significant change in height standard deviation scores (SDS) during rhGH administration. In Noonan patients, the increment in height velocity during rhGH administration was directly related to birth weight, suggesting that low-birth-weight children with Noonan syndrome responded less well to treatment. The changes in bone age, growth velocity, and height SDS were similar in Turner and Noonan syndrome groups. The authors conclude that the linear growth response to short-term administration of rhGH is comparable in patients with Noonan and Turner syndrome.

De Schepper J, et al. *Acta Paediatr* 1997;68:943-946.

Editor's comment: *Although phenotypically similar, patients with Noonan syndrome have growth patterns distinct from those of patients with Turner syndrome. The mean adult height of male patients with Noonan syndrome is 162.5 cm, and the mean adult height of female patients is 152.7 cm; the latter is almost 10 cm greater than the mean adult height of untreated subjects with Turner syndrome.[1] Romano et al[2] reported that 3/6 males with Noonan syndrome treated with rhGH achieved final heights greater than predicted, but specific data were not provided. In view of the minimal positive effect of rhGH on final height of normal short children,[3] assessment of the role of rhGH treatment in children with Noonan syndrome must be deferred until adult height data are available. Incidentally, the spontaneous growth pattern of Northern European patients with Turner syndrome recently has been reported.[4] The mean adult height of these subjects was 146.9 cm, approximately 4 cm greater than that reported by other investigators, underscoring once more the importance of ethnic as well as familial genetic factors on growth.*

Allen W. Root, MD

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3. Schmitt K, et al. *Eur J Pediatr* 1997;156:680-683.
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The Duration of Puberty in Girls Is Related to the Timing of Its Onset

The authors serially took the history of and examined 163 normal girls from age 10 to 15 years, determining the ages at which thelarche developed and menarche occurred. The mean age at menarche was 12.62 years (see Table). The younger the age at thelarche the more prolonged was the interval between thelarche and menarche. There was an inverse relationship between age at thelarche and interval to menarche.

Marti-Henneberg C, et al. *J Pediatr* 1997;131:618-621.

Editor's comment: *The investigators defined menarche not as the first episode of vaginal bleeding, but as the first menses that was followed by "regular cycles." While this definition is different than the usual one used in the United States, the data are of interest because they address the issue of the tempo of pubertal development and suggest that the later its onset, the more rapid is the progression of sexual maturation. The manuscript utilizes the term "duration of puberty" as the interval between thelarche and menarche. This is misleading as the duration of puberty extends well past this point.*

Allen W. Root, MD

Age at Menarche and the Duration of Puberty in the Overall Study Sample and the Subgroups Assigned by Age-of-Onset of Puberty

	Menarche Age		Duration of Puberty	
Study Subjects	Mean ± SEM	Range (y)	Mean ± SEM	Range (y)
Total (n = 163)	12.62 ± 0.06	10.25 - 14.41	1.96 ± 0.06	0.25 - 4.25
9 y (n = 22)	11.77 ± 0.15*	10.25 - 12.91	2.77 ± 0.15*	1.25 - 4.25
10 y (n = 53)	12.27 ± 0.10	11.00 - 13.91	2.27 ± 0.10	1.00 - 3.91
11 y (n = 54)	12.77 ± 0.07	11.59 - 14.25	1.78 ± 0.07	0.59 - 3.25
12 y (n = 27)	13.44 ± 0.10	12.42 - 14.41	1.44 ± 0.10	0.42 - 2.41
13 y (n = 7)	13.65 ± 0.09	13.25 - 13.92	0.65 ± 0.09	0.25 - 0.92

Menarche is defined as "regular cycles." Duration of puberty is defined as the period between thelarche and regular cycles.
Correlation (age at onset versus age at menarche) $r = 0.66$; $P<0.001$
Correlation (age at onset versus duration of puberty) $r = 0.62$; $P<0.001$
* Stepwise analysis of variance $P<0.001$ between groups
From Marti-Henneberg C, et al. The duration of puberty in girls is related to the timing of its onset. *J Pediatr* 1997;131:618–621.

Post-Program Self-Assessment/CME Verification

Instructions: The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. Which of the following are potential pitfalls in provocative GH testing?
   a. The arbitrary definition of a "normal" response.
   b. Interassay variations among various GH radioimmunoassays.
   c. The impact of nutrition, adiposity, and emotional state on the GH response.
   d. Age variability and sex steroid effect of the GH response.
   e. All of the above.
2. Auxologic guidelines for the diagnosis of GHD include which of the following?
   a. Severe growth retardation (-3 SD for chronologic age) in the absence of an alternative explanation.
   b. A predisposing condition, eg, cranial irradiation.
   c. Other evidence of pituitary dysfunction, eg, other pituitary hormone deficiencies.
   d. Amblyopia.
3. Dr. Rosenfeld suggests that the starting dose of GH in a younger GHD child usually should be
   a. 0.025 mg/kg/d.
   b. 0.05 mg/kg/d.
   c. 0.075 mg/kg/d.
4. Which of the following statements is/are true?
   a. The characteristic short stature in TS is of endocrine etiology.
   b. Serum concentrations of GH and IGF-1 are normal for age in prepubertal girls with TS.
   c. GH therapy in TS should be considered pharmacologic, not physiologic.
   d. All of the above.
5. In Dr. Rosenfeld's opinion, which of the following conditions have been adequately studied to be treated therapeutically with GH when short stature is present?
   a. Hypophosphatemic rickets.
   b. Idiopathic GHD.
   c. Idiopathic short stature.
   d. Dysmorphic skeletal dysplasia.
   e. Prader-Willi syndrome.
6. The recommended dose of hGH on many package inserts for treatment of GHD is
   a. 0.3 mg/kg/wk.
   b. 0.1 mg/kg/wk.
   c. 0.5 mg/kg/wk.
7. The physiologic replacement dose of hGH in a child of prepubertal age is approximately
   a. 0.3 mg/kg/wk.
   b. 0.1 mg/kg/wk.
   c. 0.5 mg/kg/wk.
8. In Dr. Slyper's opinion, which of the following complications is the one of most significant concern when using pharmacologic GH treatment?
   a. Malignancy.
   b. Slipped capital femoral epiphysis.
   c. Hyperinsulinemia and insulin resistance.
9. It is generally agreed that there is insufficient evidence to implicate GH
   a. As a cause of leukemia.
   b. As a cause of colonic polyps.
   c. As etiologic in developing atherosclerosis.
10. Dr. Slyper recommends which of the following?
    a. Treatment with hGH should be less than 0.3 mg/kg/wk.
    b. Families of individuals receiving hGH should be informed that negative short-term data provide no assurance as to the ultimate safety of pharmacologic GH therapy.
    c. Families of children with TS who are to receive treatment should be told there is no safety information on the long-term treatment of children with pharmacologic doses of hGH.
    d. All of the above.

Answer Key: 1. e 2. a,b,c 3. a 4. b,c 5. b 6. a 7. b 8. a 9. a,c 10. d

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Molecular Physiology of Leptin and Its Receptor

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INTRODUCTION

Many years of experimental physiology in rodents and humans* suggested the existence of a humoral signal "reporting" body fat mass to the brain.[1-3] Physiologic studies of rodent single-gene obesities, "*obese*" (*ob*) and "*diabetes*" (*db*) mice and Zucker *fatty* (*fa*) rats, indicated that the respective gene products play an important role in such a signaling system. The recent cloning of the *ob* (leptin; gene symbol, *Lep*) and *db/fa* (the leptin receptor; gene symbol, *Lepr*) genes have now identified some of the specific molecular components of this system for regulation of body weight. This brief review summarizes our current understanding of the molecular physiology of leptin and its receptor.

* Capital letters (*LEP* and *LEPR*) designate human genes. Small letters (*Lep* and *Lepr*) designate rodent genes.

In This Issue

Molecular Physiology of Leptin and Its Receptor ..... page 17
Letter From the Editor ..... page 17
CME Information ..... page 24
Insulin, IGF-2 and Type 1 Diabetes Mellitus: Recently Implicated Genetic Loci ..... page 27
Abstracts ..... page 32

PHENOTYPE OF *ob* AND *db* MICE

The *obese* (Lep^{ob}) and *diabetes* ($Lepr^{db}$) mutations are autosomal recessive mutations located on mouse chromosomes 6 and 4, respectively. The *ob* mutation, identified at the Jackson Laboratory in 1950, arose originally on stock V and was later bred to the C57BL/6J line, a diabetes-resistant strain.[4] The

Letter From the Editor

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For the Editorial Board,

Robert M. Blizzard, MD
Editor-in-Chief

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mutation was named "obese" because of the severe obesity of the homozygous mutant C57BL/6J mice. The *diabetes (db)* mutation was identified at the Jackson Laboratory in 1966.[5] The *db* mutation arose spontaneously on C57BL/KsJ, a diabetes-susceptible strain. The C57BL/KsJ *db/db* mice developed severe diabetes; thus, the mutation was given the name of "diabetes." *Ob/ob* and *db/db* animals are phenotypically indistinguishable when the mutations are maintained in the same strain background.[6,7] Both mutations result in hyperphagia, impaired thermoregulatory thermogenesis, and hypothalamic infertility. Homozygous affected animals develop severe and early onset obesity with reduced lean body mass, shortened axial skeleton, and decreased brain size.[3] The mutant animals also are physically less active and develop obesity even in the absence of hyperphagia.[3,8] Thus, over a period of 6 weeks, *db/db* mice pair-fed to lean controls accumulated 42% more body weight and approximately 5 times more fat mass than their lean littermates, indicating an increase of energy efficiency and preferential partitioning of calories to fat in the *db/db* mice.[8] When maintained in C57BL/6J, both *ob/ob* and *db/db* animals develop transient hyperglycemia early in life (8 to 10 weeks), but later are euglycemic and hyperinsulinemic because of compensatory pancreatic beta-cell hyperplasia and hypertrophy. When these mutations are maintained on a diabetes-susceptible strain, such as C57BL/KsJ, the mutant animals become severely diabetic with insulinopenia due to subsequent pancreatic beta-cell atrophy.[6,7] The genes that mediate such strain-specific differences in diabetes susceptibility are not known, but are clearly of great interest for the insight they will provide into the molecular bases for diabetes susceptibility.[9-11]

The functional nature of the *ob* and *db* gene products was first suggested by Coleman's parabiosis (cross-circulation) experiments, in which he joined *ob/ob* and *db/db* mice to lean partners and to each other.[3] Coleman's experiments suggested that the *ob* animal was mutant in a gene for a circulating suppressor of food intake while the *db* animal was mutant in a receptor for this hormone.

MOLECULAR CLONING AND CHARACTERIZATION OF LEPTIN

The complexity of the metabolic and endocrine phenotype of *ob* and *db* mice made it extremely difficult to identify the primary molecular defects. Hence, in the mid-1980s, projects were initiated to clone *ob* and *db* genes by positional genetic strategies.[12-15] This technique enables the cloning of genes based on their physical location on a chromosome, without prior knowledge of their precise function, and has been successfully employed for cloning cystic fibrosis, muscular dystrophy, and Huntington's disease genes.[16-18]

The mouse *ob* gene was cloned by this technique in 1994. The gene encodes a 167 amino acid precursor protein that is primarily expressed in white adipose tissues. The mature protein (named "leptin," from the Greek word "lepto," meaning "thin"), consisting of the C-terminal 146 amino acids of the precursor, is secreted into the circulation from adipocytes. Human and mouse leptin genes share approximately 85% homology at the amino acid level.[19] Both genes consist of 3 exons separated by approximately 10.6 kb and approximately 2.3 kb introns, spanning approximately 20 kb. The leptin mRNA is approximately 4.5 kb in length, with approximately 55-bp 5' untranslated region (UTR) and approximately 3.9-kb 3' UTR. The protein coding sequence is contained in the second and the third exons.[20-22]

Despite the absence of primary sequence similarity, the tertiary structure of leptin is similar to that of members of the cytokine family, consisting of 4 antiparallel α helices connected by 2 long crossover links and 1 short loop. The helical structure is highly conserved among the long-chain helical cytokine family members. The relatively loose, coil-structured crossover links and loops may provide flexibility for receptor binding-induced conformational changes, as is the case for hGH/hGHR.[23-25]

REGULATION OF LEPTIN GENE EXPRESSION

The promoter region of the human and rodent leptin genes contains a TATA box-like sequence and a functional C/EBP (CCAAT/enhancer-binding protein) alpha binding site within the 200-bp region upstream of the transcriptional start site. The C/EBP site is likely responsible for the expression of leptin in mature adipocytes since C/EBP itself is a differentiation-induced transcription factor.[26,27] Another nuclear transcriptional factor, peroxisome proliferator-activated receptor (PPAR)-gamma, which is involved in adipocyte differentiation, downregulates leptin expression by apparent antagonism of C/EBP transactivation of leptin expression.[28] The antidiabetic agent, thiazolidinedione, is a high-affinity ligand of PPAR-gamma and inhibits leptin expression in adipose tissue.[29]

Expression of leptin mRNA is increased by glucocorticoids[30-32] and insulin,[33-35] and decreased by β-adrenergic agonists and cyclic adenosine monophosphate (cAMP).[30,36] Leptin also appears to regulate its own expression. A rat mutation in the

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leptin receptor gene (*Lepr*^{fa}) results in a gene dose-dependent increase of leptin mRNA expression in adipose tissue in 10-day-old rat pups segregating for *Lepr* ^{fa}. The effect of *Lepr* ^{fa} on leptin gene expression is independent of fat mass and insulin concentration in these rats.[37] This apparent autoregulation of leptin expression may be important in mediating the pulsatile release of leptin and restricting leptin overexpression because of the potent effects of leptin on energy homeostasis and sexual development.

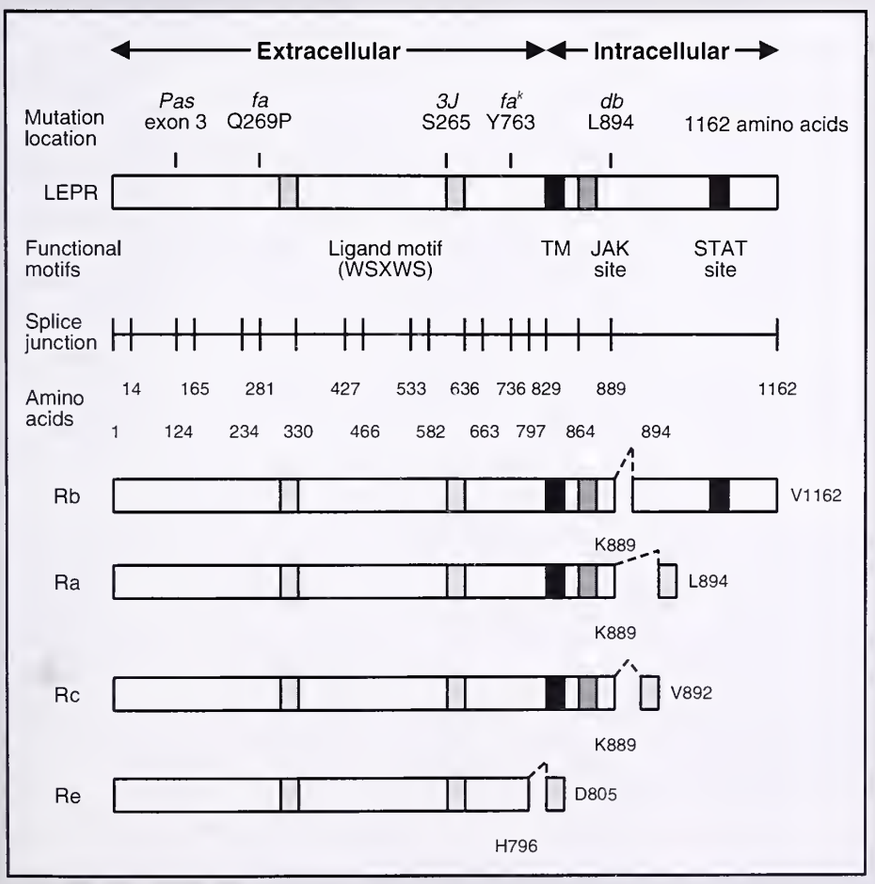
Northern blot and reverse transcriptase polymerase chain reaction (RT-PCR) analyses have shown significantly different levels of leptin gene expression among different fat depots. For example, leptin mRNA expression is higher in subcutaneous than intra-abdominal fat depots in humans.[38,39] Interestingly, the amount of intra-abdominal fat conveys a significant risk factor for obesity-related medical complications, such as diabetes and coronary artery disease. Lower leptin expression in the intra-abdominal fat depots may contribute to their adverse effects, perhaps by effects on hepatic glucose homeostasis.

Preadipocytes, such as 3T3-F442A cells, do not express leptin.[34] Mature adipocytes, differentiated from 3T3-F442A cells in culture, express very low levels of leptin mRNA (1% of that in epididymal adipose tissue).[35] However, mature adipocytes derived from subcutaneously implanted 3T3-F442A cells express leptin mRNA at levels about 15% of those in epididymal adipose tissue. These data suggest that factors required for high level expression of the leptin gene in vivo are lacking in the cell culture system.[40] Identification of these factors would shed important light on the in vivo regulation of leptin expression.

MOLECULAR CLONING AND CHARACTERIZATION OF THE LEPTIN RECEPTOR

The leptin receptor gene was cloned by screening a cDNA expression library of choroid plexus using a leptin-alkaline phosphatase fusion protein as a probe. The receptor has a high affinity for leptin (kd $\cong 0.7\times10^{-9}$ M).[41] This gene was quickly demonstrated to be mutant in the *db* mouse and the *fa* rat.[42-44] The leptin receptor gene encodes a membrane protein that shares 24% sequence homology with gp130, a signal-transducing component of the

Figure 1



Schematic of functional domains, mutations, and splice variants of the mouse and rat leptin receptor (LEPR). The extracellular domain of the receptor is encoded by exons 1 through 15, the transmembrane domain by exon 16. The intracellular domain is encoded by exon 17 and either terminal exon 17', 18a, or 18b for the Rc, Ra, and Rb isoforms, respectively.[43,45] Exons 3 through 6 and 8 through 11 encode two potential leptin binding sites, containing the characteristic four conserved cysteine residues and the Trp-Ser-X-Trp-Ser motif (where X is a nonconserved amino acid). The second binding site appears to be important for leptin binding and signaling.[120] The transmembrane (TM) domain is 23 amino acids long. The Janus kinase (JAK) and STAT protein binding sites are encoded by exons 17 and 18b, respectively. The splice variants Rb, Ra, Rc, and Re have been described in at least two species. The Rd and Rf (not shown) have been identified in only one species: Rd in mouse, and Rf in rat.

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interleukin-6 receptor. The extracellular domain of the leptin receptor contains all of the characteristically conserved cysteine residues, the Trp-Ser-X-Trp-Ser motif, and other conserved residues characteristic of the cytokine receptor superfamily (Figure 1, page 19). The large extracellular domain of the receptor (approximately 865 amino acids) is followed by a single transmembrane domain (23 amino acids) and cytoplasmic domains of varying length (up to 273 amino acids) resulting from alternative splicing of the C-terminal exons.[41,45] Of the 6 alternative splice variants of LEPR described, 4 have been detected in at least 2 different species (Figure 1, page 19).[45] The longest isoform, "Rb" (1162 amino acids), is expressed predominantly in the hypothalamus, and at very low levels in many other tissues, including adipose tissue, skeletal muscle, pancreas, and liver. The "Rb" isoform of LEPR contains a Janus kinase (JAK) binding site (FWDDVPNP motif) and STAT binding site (YMPQ motif). JAK, a class of cytoplasmic tyrosine kinase, and STAT (signal transducer and activator of transcription, a family of latent cytoplasmic transcription factors) participate in signal transduction for members of the cytokine superfamily.[46,47] The "Rb" isoform of LEPR is the only isoform apparently not produced in the *db* mouse and is thereby shown to be essential for mediating leptin's effects on energy homeostasis.[43,44] A shorter isoform of LEPR, "Ra" (894 amino acids), is expressed at relatively higher levels in choroid plexus, kidney, and lung, and at lower levels in liver, muscle and adipose tissue.[41,43] By RT-PCR, mRNAs for the "Rc" isoform, and a putative soluble isoform "Re," also were detected in adipose tissue and heart.[43] The biologic functions of the shorter splice variants of LEPR are not clear, but may involve binding leptin molecules in the circulation and/or transport of leptin across cell membranes.

The leptin receptor gene includes 20 exons (18 coding), spanning about 70 kb.[45,48] Human and mouse leptin receptor genes share approximately 78% homology at the amino acid level.[41]

MUTATIONS OF *Lep* AND *Lepr* IN RODENTS

All known spontaneous mutations in the *Lep* and *Lepr* genes in rodents have been recently characterized at the molecular level.[19,43,44,49-55] These results are summarized in Table 1 and Figure 1. Both the *ob* and ob^{2J} mutations result in little or no detectable leptin protein in the circulation. The *db* and *fa* mutations result in functional disruption of 1 or more of the splice variants of the leptin receptor. The mutant animals are functionally leptin-resistant and have higher plasma leptin concentrations per unit of fat mass than lean controls, presumably resulting in part from derangement of the apparent receptor-mediated autoregulation of leptin expression in adipose tissue.[37]

Studies of the *db* mutant confirmed the functional importance of the longest isoform ("Rb") of the receptor. The *db* mutation, which abolishes the "Rb" isoform of the receptor without affecting any of the other known splice variants, results in an obesity phenotype that is indistinguishable from that seen in null mutations, such as db^{Pas}, db^{3J}, and fa^{k}, which affect all known splice variants of *Lepr*.[42,54,55] The fact that the "Rb" isoform contains the complete JAK/STAT signaling motifs also is consistent with the apparent essentiality of this splice variant.

MOLECULAR GENETICS OF *LEP* AND *LEPR* IN HUMANS

The human leptin gene (*LEP*) is located on chromosome 7q31.3;[56] the human leptin receptor gene (*LEPR*) maps to chromosome 1p31-p22.[57] A single guanine nucleotide deletion in codon 133 of *LEP* that leads to a frameshift was recently found in 2 obese cousins in a highly consanguineous family of Pakistani origin living in England. These children, an 8-year-old female (86 kg) and a 2-year-old male (29 kg), have very low plasma concentrations of a leptin molecule that is probably inactive (Table 1).[58] However, except for their extreme obesity, the gross phenotype of these children differs significantly from the *ob/ob* mouse in that they do not display apparent derangement of thermoregulation, overproduction of cortisol, or stunting in height (which may be mediated by the effects of excessive glucocorticoid in the mice). Hypothalamic hypogonadism would be expected, although these children are still prepubertal and the gonadal axis has not been formally assessed. A role for leptin in human energy homeostasis is confirmed by the obesity of these children.

A second mutation in the leptin gene (*LEP*), and the first mutation in the leptin receptor gene (*LEPR*) have been recently described in two families (Table 1).[59,60] The homozygous affected individuals in these families are hyperphagic and extremely obese. Hypogonadism and the absence of sexual maturation are common features in the adult patients. As predicted by the phenotype of *Lepr* mutant rodents, the humans homozygous for the *LEPR* mutation show evidence of autonomic nervous system dysfunction.

Significant coding sequence variants in *LEP* are extremely rare in humans.[61-64] However, extreme obesity in humans (BMI [body mass index=wt (kg)/ht (m^2)] >42 from a US population; and BMI >35 from a French population) has been associated by genetic linkage analysis with the vicinity of the leptin

gene.[65,66] Some obesity-related traits, such as skinfold thickness and serum proinsulin concentration in Mexican-Americans, also have been associated with the genetic region of the leptin gene.[67] Since significant variations in the coding sequence of the leptin gene have been reported only in the Pakistani family described above, these linkages could be due to variations in the noncoding region of *LEP* or could be due to another obesity gene in this region.[59-62]

Several nonconserved amino acid substitutions in the coding exons of the *LEPR* gene also have been identified in humans.[68-71] Sequence variations in the noncoding region of *LEPR* have been linked to acute insulin response to intravenous glucose tolerance testing (IVGTT) in Pima Indians, who have a very high prevalence of obesity and noninsulin-dependent diabetes mellitus (NIDDM).[48,71] Linkage between a marker in the leptin receptor region and BMI, body composition, and plasma insulin concentration also were observed in the Quebec Family Study.[72] Thus, these less drastic variations in coding sequences of *LEP* or *LEPR*, with attendant subtle alterations in leptin signaling, or variations of the promoter region that affect the regulation of *LEP* and *LEPR* gene expression may predispose individuals who carry these allelic variations to obesity.

BIOLOGIC EFFECTS OF LEPTIN IN RODENTS

Intraperitoneal or intracerebroventricular (ICV) administration of recombinant leptin to *ob/ob* mice reverses virtually all the characteristics of the phenotype. Leptin decreases food intake, increases energy expenditure, including thermoregulatory

Table 1
Spontaneous Mutations in the Leptin and Leptin Receptor Genes in Rodents and Humans

Mutations or Gene Symbol	Species	Mutant Molecule	Nature of Mutation	Result of Mutation
ob	mouse	leptin	C→T mutation in the last exon (Arg105Stop)	No detectable leptin protein, but high level of mutant leptin mRNA[19]
ob^{2J}	mouse	leptin	Transposon insertion in the first intron; abnormal splicing; no leptin mRNA	No detectable leptin mRNA and protein[49]
db	mouse	leptin receptor	G→T mutation in the 3'UTR of "Ra" creates a splice donor site; insertion of first 106 bp of the last "Ra" exon into "Rb" mRNA	No "Rb" isoform; all other splice variants are normal[43,44]
db^{3J}	mouse	leptin receptor	17-bp deletion in exon 11; frameshift (start at Ser625)	No leptin receptor mRNA[54]
db^{Pas}	mouse	leptin receptor	Duplication of exons 3-6; frameshift	No leptin receptor mRNA[55]
fa	rat	leptin receptor	A→C mutation in exon 5 (Gln269Pro)	Reduced surface expression of the mutant leptin receptor; no effect on affinity for leptin[50,51]
fa^{k}	rat	leptin receptor	T→A mutation in exon 14 (Try763Stop)	No leptin receptor mRNA[52,53]
LEP	human	leptin	A single guanine nucleotide deletion at codon 133; frameshift	Little or no detectable leptin protein[58]
LEP	human	leptin	C→T mutation in the last exon (Arg105Trp)	Mutant protein produced but not secreted[59]
LEPR	human	leptin receptor	G→A mutation in the splice donor site of exon 16	Truncation of leptin receptor after exon 15 with an extra Glu residue at the C-terminus[60]

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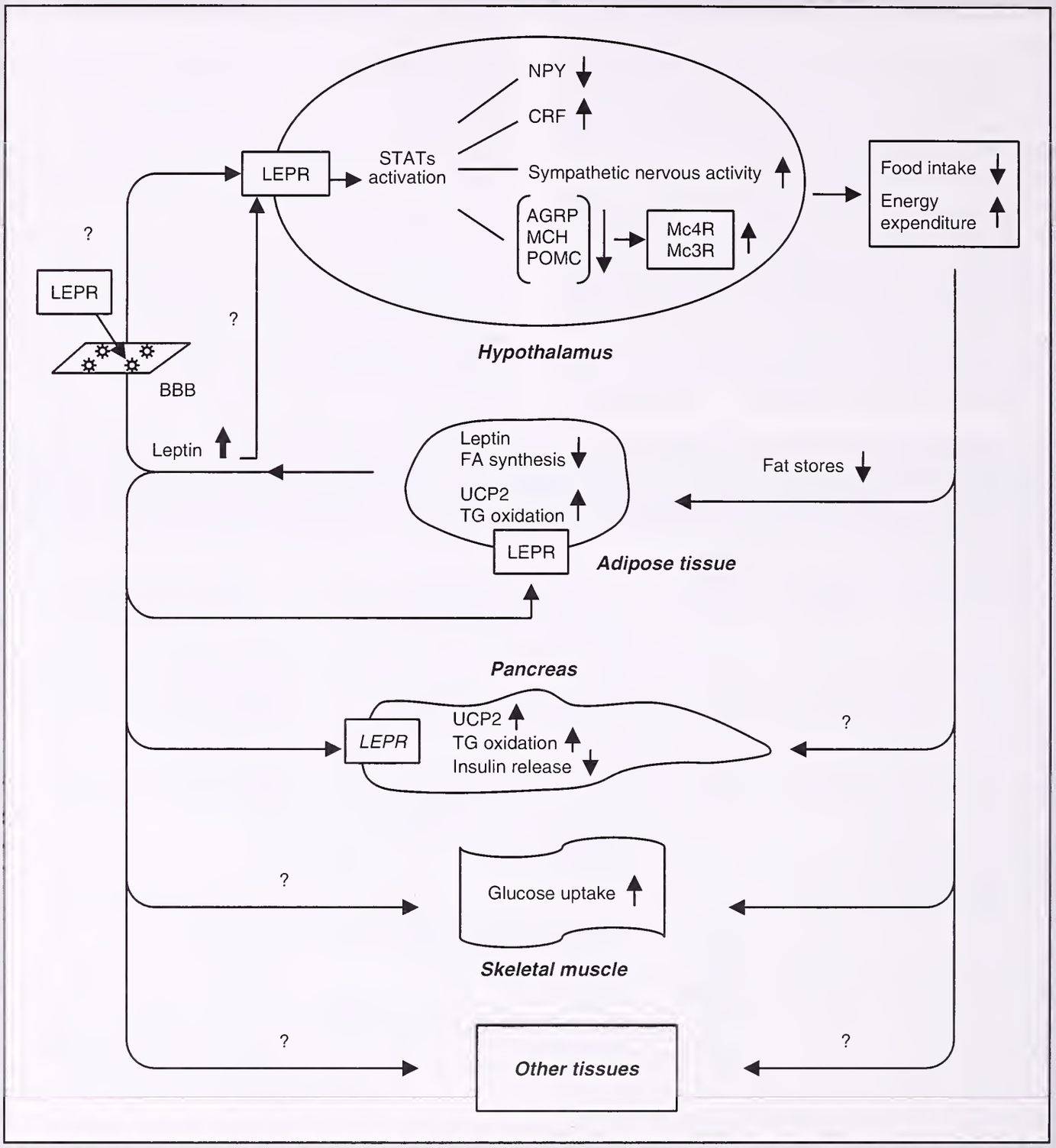
Figure 2



Schematic of the current view of the role(s) of leptin in energy homeostasis. Leptin is released by adipose tissue and circulates in the plasma. The concentration of leptin in plasma is very closely correlated with the amount of body fat. Plasma and/or CSF leptin molecules bind to a receptor (LEPR) in the hypothalamus, which triggers (via JAK/STAT) a series of changes of gene expression of several neuropeptides, including downregulation of NPY and upregulation of CRF and AGRP, and increases of sympathetic nervous activity. These changes result in decreased food intake and increased energy expenditure, and eventually lead to weight loss. Leptin also acts directly on peripheral tissues, regulating uncoupling proteins (UCPs) and leptin gene expression, fatty acid (FA) synthesis, and triglyceride (TG) oxidation. The decline of circulating leptin associated with reduced fat mass and/or hypocaloric intake leads to the opposite effects, triggering increased food intake and conservation of energy, which favors storage of body fat.

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thermogenesis and physical activity, and causes rapid weight loss (mainly adipose tissue loss) in the *ob/ob* mouse.[73-77] The effects of leptin on energy metabolism are summarized in Figure 2. Administration of leptin also decreases blood glucose and insulin concentrations in *ob/ob* mice,[78] and acutely stimulates glucose turnover and glucose uptake by skeletal muscles and decreases liver glycogen content in lean mice.[79] As predicted, administration of leptin has no effect in *db/db* mice.[74,75]

Leptin apparently affects food intake and energy expenditure via direct actions on peripheral tissues as well as effects mediated by the central nervous system (CNS). Leptin induces depletion of triglyceride in adipose tissue and pancreas by increasing intracellular fatty acid oxidation and gene expression of the enzymes involved in fatty acid oxidation.[80,81] Leptin also increases the expression of uncoupling protein-2 (UCP2) in adipose tissue and pancreas, which may partly account for the increase in energy expenditure after leptin administration.[81] These effects of leptin on adipose tissue and pancreas are apparently mediated by the leptin receptor in these tissues since the same effects are observed in both intact animals and isolated tissues. The effects are not observed in tissues isolated from *db/db* mice, or in *fa/fa* rats, suggesting the "Rb" isoform of the receptor is responsible for mediating these effects.[81] Leptin apparently also inhibits basal and glucose-stimulated insulin secretion by direct effects on pancreatic beta cells.[82,83]

CNS-MEDIATED EFFECTS OF LEPTIN

The CNS-mediated effects of leptin on food intake and energy expenditure are mediated by specific neurons in the hypothalamus. The "Rb" isoform of the leptin receptor is primarily expressed in the arcuate, ventromedial, dorsomedial, and lateral hypothalamic nuclei,[84] which have been previously implicated in energy homeostasis.[85] The efferent signals from these neurons, affecting food intake and energy expenditure, are then "communicated" to peripheral tissues via the neuroendocrine and autonomic nervous systems.

The expression levels of several hypothalamic neuropeptides are influenced by leptin administration, and these neuropeptides may in turn mediate a portion of leptin's effects on energy intake and expenditure. One of these neuropeptides is neuropeptide Y (NPY). Central administration of NPY potently stimulates food intake, decreases sympathetic nervous activity, and increases plasma insulin and corticosterone concentrations.[86] ICV administration of leptin inhibits the expression and release of NPY in the arcuate region of the hypothalamus in *ob/ob* mice.[87,88] Knockout of the *Npy* gene partially reduces the obesity phenotype of *ob/ob* mice.[89] However, *Npy* knockout mice are still sensitive to the anorexic effects of leptin,[90] suggesting the existence of NPY-independent downstream effectors of leptin action and food intake. ICV administration of leptin also increases the expression of corticotropin-releasing factor, a potent inhibitor of food intake, by 30% in the paraventricular nucleus of *ob/ob* mice.[88] Other possible candidates for downstream mediators of leptin effects include melanin-concentrating hormone,[91] agouti-related protein,[92,93] and urocortin,[94] all of which have been implicated in the regulation of ingestive behavior and/or energy expenditure in animals.

The CNS-mediated effects of leptin also are reflected in its critical role in normal reproductive function. Since ovaries of *ob/ob* and *db/db* mice function normally in wild-type host after ovarian transplantation, the infertility of *ob/ob* mice is apparently due to the neuroendocrine defect in the gonadal axis and not to a cell-autonomous defect in the ovary.[95,96] Leptin administration restores the reproductive function in both female and male *ob/ob* mice[97,98] and induces vaginal opening in prepubertal mice, indicating that plasma leptin concentration may be a signal for the onset of sexual maturity and maintenance of fertility.[99]

Fasting results in a rapid decline in circulating leptin concentration, and this decline appears to mediate some of the changes in gonadal, adrenal, and thyroid neuroendocrine axes induced by fasting. Short-term restriction of food intake in mice results in lower serum triiodothyronine and thyroxine levels and increased corticotropin and cortisol levels, concomitant with the decline in plasma leptin concentration. Repletion of exogenous leptin during the short-term starvation restores circulating triiodothyronine and thyroxine levels, reduces circulating cortisol levels, and prevents the starvation-induced delay of ovulation in female mice.[100]

SIGNAL TRANSDUCTION OF LEPTIN/LEPTIN RECEPTOR

The structural similarities between leptin and members of the class I cytokine family suggest a similar mode of ligand-receptor binding and signal transduction for leptin and the other members of the family, including interferons, interleukins, growth hormone, and insulin-like growth factor 1 (IGF-1).[23,41,46] Binding of a ligand to this class of receptor triggers assembly of receptor components or dimerization of receptor molecules, and activates JAK, which is

bound to the cytoplasmic domain of the receptor. The activated JAK then phosphorylates tyrosine residues on the cytoplasmic domain of the receptor, which activates binding sites for the Src homology 2 group of latent cytoplasmic proteins, STATs. The phosphorylated STATs form homodimers and/or heterodimers, translocate into the nucleus, and participate in transcription regulation by binding to a specific STAT response element in the target genes.[47] Several STAT proteins have been implicated in leptin signaling. STAT 3 was shown to be phosphorylated in the hypothalamus of *ob/ob* mice but not *db/db* mice after systemic leptin administration.[101] STATs 1, 3, 5B, and/or 6 were reported to be phosphorylated upon leptin stimulation in *LEPR*-transfected COS cells and GT1-7 (a hypothalamic cell line) cells.[102-104] All these STAT proteins also have been shown to be involved in the signal transduction of many other members of the cytokine family. Leptin increases the association of phosphatidylinositol 3 (PI3) kinase with insulin receptor substrate (IRS)-1 and -2,[105,106] which are involved in signal transduction of insulin, cytokines, and growth factors. How the specificity of leptin signaling is defined by the activation of these STAT proteins and the changes of intracellular signaling molecules such as PI3 kinase and IRSs has yet to be worked out.

In addition, the possibility that the "Ra" isoform (Figure 2, page 22), which has a JAK binding site but no STAT binding domain, in combination with other molecules also is involved in the signal transduction of leptin cannot be ruled out. Although the "Ra" isoform does not induce phosphorylation of STATs 1, 3, and 5B in transfected COS cells,[103] this isoform has been reported capable of inducing expression of immediate early genes *c-fos*, *c-jun*, and *jun-B*, in transfected COS cells after leptin stimulation.[107]

PLASMA LEPTIN CONCENTRATIONS IN HUMANS

Plasma leptin concentrations are highly correlated with total fat mass in humans (r=0.95).[108] The concentration in plasma ranges approximately from 2 to 20 ng/mL in normal weight individuals, and 10 to 300 ng/mL in obese individuals. A single regression line relating fat mass to circulating leptin concentration fits both obese and nonobese subjects of the same sex, suggesting that the plasma leptin level is similarly regulated in both obese and lean individuals. In humans, plasma leptin concentrations per unit fat mass are 2- to 3-fold higher in females than in males.[108] These differences may be due in part to the higher percentage of body fat in subcutaneous depots in females. This depot has higher leptin mRNA expression than intra-abdominal fat depots, especially in females.[38,39] In addition, the differences of ambient gonadal steroids also may play a role in the sexual dimorphism of leptin expression in humans. Premenopausal women have higher plasma leptin concentrations than postmenopausal women. In premenopausal women, serum leptin levels are higher in the luteal phase than in the follicular phase,[108,109] suggesting that estrogen and/or progesterone may increase leptin gene expression. On the other hand, testosterone replacement normalizes elevated serum leptin concentrations in hypogonadal males,[110] suggesting that androgens may suppress leptin expression.

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

LEPTIN RESISTANCE

The higher plasma leptin concentrations in obese humans indicate that leptin deficiency is not a common cause of obesity in humans. Thus, it has been suggested that the obese may be leptin-resistant. The mechanism of such apparent leptin resistance is not fully understood, but might be due to: (1) A defect in leptin signal transduction and the function of downstream signal mediators. As in the *db/db* mouse and *fa/fa* rat, mutations in the leptin receptor will obviously impair leptin signal transduction and cause leptin resistance. A null mutation in the leptin receptor gene and some subtle variations in both coding and noncoding sequences of the *LEPR* gene have been identified in humans.[48,69,72] These mutations of *LEPR* or mutations of the downstream mediator genes may reduce the intensity of leptin signal. Thus, a higher leptin concentration (therefore higher fat mass) would be required in these individuals to elicit an intensity of leptin signal equal to that in normal weight individuals. As a result, these individuals would appear to be leptin-resistant and obese (ie, have a higher "set point" of body weight); (2) Limited transport of leptin from plasma to cerebrospinal fluid (CSF). Since many arcuate neurons project axons to the median eminence, which is exposed to blood circulation directly, it is not clear whether leptin must cross the blood-brain barrier to act upon the CNS. However, rodents are more sensitive to ICV than to peripheral administration of leptin.[73] In obese humans, CSF leptin concentrations are disproportionately low relative to plasma leptin concentrations, although absolute concentrations of leptin in CSF are slightly higher than those in normal weight subjects. The ratio of CSF [LEP]:plasma [LEP] decreases as plasma leptin concentration increases, suggesting that there is a saturable transport of leptin from plasma into CSF. The limited transport of leptin from plasma to CSF could be one of the factors contributing to apparent leptin resistance in human obesity.[111,112]

MOLECULAR PHYSIOLOGY OF LEPTIN IN HUMANS

Plasma leptin concentrations in humans show a diurnal rhythm, with the lowest concentrations occurring around noon to mid-afternoon and the highest concentrations occurring around midnight to early morning.[113] Plasma leptin concentrations also show ultradian oscillations, with a frequency of approximately 43.8 minutes. The frequency of pulsatility of plasma leptin concentration is similar between obese and nonobese individuals. The higher plasma leptin concentration in the obese is due solely to increased leptin pulse height.[114] The humoral or neural signals that regulate the oscillations of the plasma leptin concentration are not clear at present. Autoregulation of leptin gene expression in adipose tissue by autocrine effects of leptin itself is one possibility.[37] The fluctuations of plasma leptin concentration are inversely related to those of adrenocorticotropic hormone (ACTH) and cortisol. This finding, and reports that leptin inhibits corticotropin-releasing factor expression in the hypothalamus and substantially blunts the stress- and fasting-induced rise in ACTH and corticosterone[100,114,115] suggests that leptin suppresses the hypothalamic-pituitary-adrenal axis. Therefore, leptin-mediated alterations in ACTH and cortisol secretion or action are possible mechanisms by which a peripheral signal of nutritional status (leptin) could regulate CNS production of neuropeptides that modulate endocrine function and behavior.[114]

Leptin apparently circulates in the plasma in both free and bound forms. Multiple leptin-binding proteins have been detected by gel filtration and sucrose gradient centrifugation. The ratio of free to bound leptin is lower in obese (21.4%) than in lean (46.5%) subjects, although the absolute concentration of free leptin is higher in the obese. The molecular weights of these putative leptin-binding proteins range from 80 to 240 kd.[116,117] Only about 10% of bound ^{125}I-leptin in human plasma is immunoprecipitable with leptin receptor antibodies, suggesting that other proteins besides a soluble form of the leptin receptor molecule also bind leptin in the circulation.[117]

Short-term fasting substantially reduces the plasma concentration of free leptin and decreases the ratio of free leptin to bound leptin. In humans, 24-hour fasting lowers the free plasma leptin from 19.6 ng/mL to 1.3 ng/mL in lean subjects and 28.3 ng/mL to 14.7 ng/mL in obese subjects.[117] Maintenance of a hypocaloric diet (800 calories/d) reduces total plasma leptin concentration to approximately 50% of its concentration relative to weight-stable subjects with the same fat mass.[118,119] However, overfeeding does not increase leptin expression beyond that expected for the increase of fat mass.[120]

These effects of short-term fasting and overfeeding on plasma leptin concentrations, together with the biologic effects of leptin on food intake and energy expenditure, are most consistent with a physiologic model in which leptin has evolved primarily to keep body fat mass (energy reserves) at or above a set point. Caloric restriction threatens this energy reserve, triggering an immediate decrease in plasma leptin concentration, which in turn triggers a series of neuroendocrine changes designed to

increase food intake and decrease energy expenditure. Moderate increases in fat mass, with attendant increases in circulating leptin, probably have relatively small effects on this system of energy homeostasis. Larger increases, resulting from greater increases in fat mass or the exogenous administration of leptin, result in decreases in food intake and increases in energy expenditure.

This model of leptin physiology suggests that exogenous leptin may be useful in helping the formerly obese to maintain a reduced body weight by providing a leptin "signal" equal to that which preceded weight reduction.[121]

CONCLUSIONS

The primary physiologic role of leptin appears to be as a regulator of energy homeostasis by providing a signal to the CNS regarding the size of energy (fat) stores. This signal mediates changes in behavior and metabolism that tend to maintain body fat at a level determined by genetic, developmental, and environmental factors. The defense against lowered fat mass is much stronger than that against increased body fat. Evolutionary and experimental arguments support this conclusion. The major effects of leptin on energy metabolism are schematized in Figure 2 (on page 22).

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Insulin, IGF-2 and Type 1 Diabetes Mellitus: Recently Implicated Genetic Loci

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Recently, great strides have been made in elucidating the genetic components of insulin-dependent diabetes mellitus (IDDM), one of the best examples of a multifactorial disease with both environmental and polygenic etiologies. This article focuses on one of the more recently implicated and best investigated loci to date, *IDDM2*. While its effects are no doubt less than that of the major histocompatibility complex, human lymphocyte antigen (HLA), *IDDM2* maps to chromosome 11p15.5, where at least 2 candidate genes are found, those for insulin (*INS*) and for insulin-like growth factor 2 (*IGF2*).

Familial clustering and a high concordance rate in monozygotic twins indicate that genetic transmission of susceptibility is responsible for about half of the risk for the development of type 1 diabetes. The importance of the HLA locus, located on chromosome 6p21 (*IDDM1*), first came to light in the 1970s following association and linkage studies in affected and nonaffected siblings, and appears to account for 42% of the genetic component.[1] Type 1 diabetes is primarily a sporadic disease (90%); population-based (case-control) studies provided the early HLA association data. Family studies in which there were more than 1 affected child confirmed the association of specific HLA haplotypes (alleles) with type 1 diabetes (ie, the demonstration of linkage disequilibrium) and revealed preferential sharing of certain HLA haplotypes among affected sibs. The underlying biology of this linkage is not yet completely understood. It appears that the class II HLA molecules affect the immune response because of their highly polymorphic sequence variations, resulting in differences in the peptide-binding groove used in antigen presentation.[2]

That a locus involved in cellular immune recognition was involved in an autoimmune disease came as no surprise. However, 2 key questions remained: What other genes could play a role and how do we go about identifying these genes? Whereas reverse genetics (determination of the chromosomal location and identification of new genes in spite of ignorance of the disease mechanism) has resulted in spectacular successes in identifying genes responsible for single-gene (mendelian) diseases, the application of this method to common polygenic phenotypes involves difficulties whose magnitude is hard to even estimate.[3] To date, no *novel* gene has been identified and cloned on the basis of its linkage to a complex (multifactorial) disease phenotype. Linkage can only narrow the locus to within several centimorgans (cM). In human genetic maps, 1 cM roughly corresponds to 1 million bp of DNA (1 Mb) and contains, on average, about 20 genes. Furthermore, positional cloning of mendelian disorder genes relies on the occasional patient with a large deletion/insertion or chromosomal rearrangement to further narrow the disease locus; this is not an option in complex disorders like diabetes, in which disease susceptibility is encoded not by gene-inactivating mutations but by subtle DNA sequence variants common in the general population.

Global searches of the whole genome, using hundreds of equally spaced microsatellite markers to detect linkage to specific chromosome locations, help to orient our search for disease-related candidate genes based on our knowledge of their participation in particular biochemical or cellular pathways.[4,5] While these studies are fraught with the statistical hazards of multiple comparisons and other methodologic controversies, they may help guide our quest for candidate genes. A disease is assigned to a chromosomal locus only after linkage has been formally demonstrated, replicated, and confirmed in at least 3 different datasets. The robustness of the linkage must be continually verified in additional datasets—preferably ones comprised of genetically homogeneous populations—and these regions need to be further saturated with markers to further define the loci. There are then association-based tests that take advantage of linkage disequilibrium (that is, the preferential association of specific marker polymorphisms with the disease) to narrow the locus to specific gene(s).

The presence of genes whose products are functionally related to the phenotype (candidate genes) in the region identified by linkage analysis can greatly accelerate this process. If no such genes exist, or if they are tried and ruled out, RNA sequences transcribed from the genetically defined interval (positional candidates) can be identified using transcript

maps of the human genome, such as the prototype recently published.[6] After the genes are fully cloned, polymorphisms in and around them can be identified and examined for association with a specific disease, eg, for alleles that are more frequent in diabetic than in nondiabetic subjects. However, the sequence hypervariability seen at *IDDM1*—the class II HLA locus—is the evolutionary result of adaptation to a wide variety of pathogens and is unlikely to be found in other functionally significant proteins. In most cases, subtle coding or even noncoding variants have to be examined for biologic significance. Table 1 presents a summary of loci detected by genome-wide scans for type 1 diabetes susceptibility. Linkage disequilibrium studies for *IDDM2* narrowed the locus to a variable number of tandem repeat (VNTR) polymorphisms immediately upstream of the gene for insulin; this was facilitated by an intense and systematic search for markers flanking the *INS* gene on chromosome 11p15.5. The importance of this region is well known because of its involvement in diseases other than type 1 diabetes (many tumors, including Wilms' tumors and adrenocortical tumors, and Beckwith-Wiedemann syndrome; see previous reviews in *GGH* 1994;10(1):1-4,6-10. Unfortunately, the current human genome map, in general, does not afford this high degree of marker density.[7-9]

THE *IDDM2* LOCUS

Researchers suspected that insulin (acting as an antigen triggering autoimmune destruction of the pancreatic beta cell) may be the product of a candidate gene for type 1 diabetes, and linkage of this disease to a locus mapping to chromosome 11p15.5 (*IDDM2*) was established a decade ago (Figure 1). More recently, thorough association studies narrowed the locus to a polymorphism 356 bp upstream of the insulin gene (*INS*) promoter consisting of a VNTR of a 14-bp consensus sequence. A large number of alleles can be distinguished by size and by different variants of this consensus repeat unit. Most alleles in whites contain either 30 to 45 repeats (class I) or, less frequently, more than 150 repeats (class III). Intermediate (class II) alleles are rare.

Approximately 40% to 45% of whites have at least 1 class III allele, compared with <20% in diabetic patients. This suggests that class III (long) alleles are protective. This protective effect is observed in I/III heterozygotes and is, therefore, dominant. The relative risk for development of type 1 diabetes is increased 3- to 4-fold when a subject is homozygous for I/I (I/I versus I/III or III/III), and this specific polymorphism is estimated to account for 10% to 15% of the familial clustering of diabetes.[10]

Table 1
Type 1 Diabetes Susceptibility Loci in Humans*

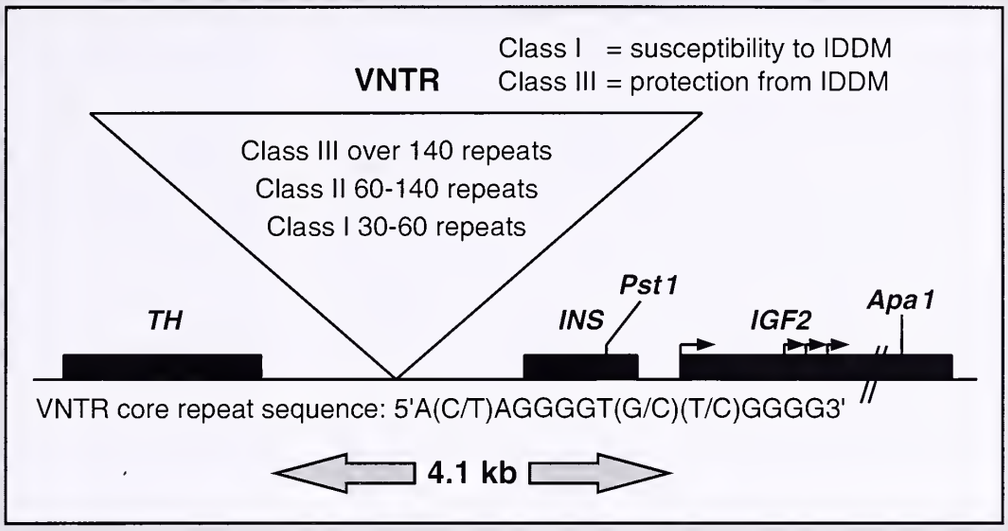
Locus	Chromosomal Location	Detected in Davies Genome-Wide Scan?[5]	Detected in Other Datasets?†
IDDM1 (HLA)	6p21	Yes	Yes
IDDM2 (*INS* 5' VNTR)	11p15.5	Yes	Yes
IDDM3	15q26	Yes	Yes
IDDM4	11q13	Yes	Yes
IDDM5	6q25	Yes	Yes
IDDM6	18q21	Yes	Yes
IDDM7	2q31	No	Yes
IDDM8	6q25-q27	Yes	Yes
IDDM9	3q21-q25	No	Cited in Todd[4]
IDDM10	10p11.2-q11.2	Yes	Yes
IDDM11	14q24.3-q31	No	Yes
IDDM12 (*CTLA-4*)	2q33	No	Yes
IDDM13	2q34	No	Yes
IDDM15 (distinct from HLA)	6p21	No	Yes
Not assigned (*GCK*)	7p	No	Yes
Not assigned	Xq	No	Cited in Todd[4]

The *IDDM* nomenclature is officially assigned to a locus after linkage has been formally demonstrated, replicated, and confirmed in at least 3 independent datasets.

† References to specific loci can be obtained by consulting *Online Mendelian Inheritance in Man* (http://gdbwww.gdb.org/omim).

Adapted with permission from Todd JA. *Proc Natl Acad Sci USA* 1995; 92:8560-8565.

Figure 1
The *IDDM2* Locus



The *IDDM2* locus, located on human chromosome 11p15.5, has been mapped to a variable number of tandem repeat sequences lying 3' to the tyrosine hydroxylase gene (*TH*) and 5' to the insulin gene (*INS*) and its close (less than 2 kb) neighbor, the gene for insulin-like growth factor 2 (*IGF2*), whose 4 promoters are indicated by the arrows. Useful RFLP exon polymorphisms to study allele-specific transcription are noted for *INS* (*Pst1*) and *IGF2* (*Apa1*). Note that the *IGF2* gene is expressed from the paternal gene copy in most tissues, whereas the other imprinted genes at the 11p15.5 locus (not shown) are expressed by the maternal gene copy (these include centromeric to *TH*: *p57KIP2* and *ASCL2*; telomeric to *IGF2*: *H19*).

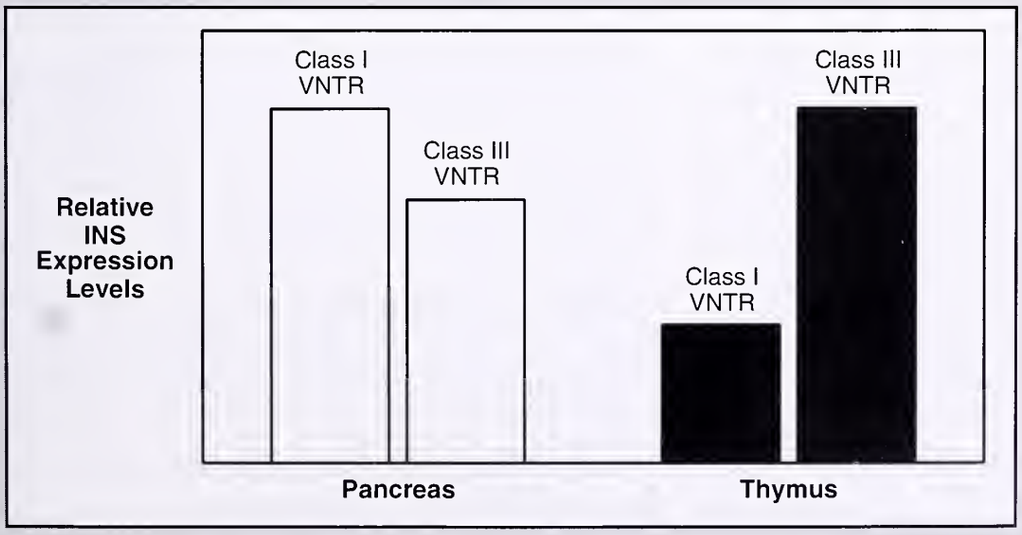
FROM GENETICS TO GENE FUNCTION

What biologic mechanism might account for this effect of the VNTR on diabetes susceptibility? Since the *INS* VNTR does not encode a protein sequence, it must exert transcriptional effects on nearby genes. Transcriptional effects of VNTRs elsewhere in the genome have been found, eg, those reported for *HRAS*[11] (Harvey rat sarcoma virus proto-oncogene), a membrane-associated small guanosine triphosphate (GTP)-binding protein believed to participate in the development and/or maintenance of certain malignancies. Although *INS* may be the principle target of its 5' VNTR, this VNTR also could affect transcription of *IGF2,* the gene encoding insulin-like growth factor 2, whose promoters are 5 to 24 kb downstream (Figure 1), a distance compatible with enhancer effects. Therefore, elucidation of the *IDDM2* mechanism requires a systematic search for allelic effects on transcription levels of genes that are in close proximity to the VNTR in tissues of known importance in diabetes.

INS AS A CANDIDATE GENE FOR *IDDM2*

Recent studies have demonstrated that the VNTR does indeed exhibit tissue-specific allelic effects on *INS* transcription in vivo and in vitro (Figure 2).[12-17]

Figure 2



Schematic summarizing VNTR effects on *INS* mRNA levels in fetal tissue, genotyped for allele class. Data for fetal pancreas are adapted from Vafiadis et al[15]; those for thymus are adapted from Vafiadis et al.[16]

Since the pancreas is the major tissue (and initially thought to be the only tissue) in which physiologically significant levels of insulin are produced, it was an important tissue to examine. To distinguish between mRNA from each gene copy in heterozygous samples of human fetal pancreas, a transcribed *Pst1* polymorphism within the 3' untranslated region of *INS* was used. By using this technique, pancreatic *INS* mRNA levels were found to be 15% to 20% lower from chromosomes bearing the class III VNTR than from those bearing the class I VNTR, easily genotyped by polymerase chain reaction (PCR).[15] Similar findings have been reported by others in adult pancreas.[12] Although statistically significant, this marginal loss of function is unlikely to account for the protective effect of class III VNTR, whose dominant nature suggests gain of function.

Mouse thymus expresses insulin[18] and many otherwise tissue-specific proteins (such as peptide hormones and exocrine pancreatic enzymes), presumably for the purpose of immune tolerance development. Human thymus also expresses insulin as mRNA and protein, albeit at levels 10^3 to 10^4 times lower than those in the pancreas. These observations prompted a search for *INS* VNTR allele-specific effects on thymic *INS* mRNA levels in 12 samples of human fetal thymus that were heterozygous at the VNTR (I/III) and informative for the transcribed *Pst1* polymorphism. Unlike pancreas, the thymic insulin mRNA value from VNTR class III haplotypes was 2- to 3-fold higher than from class I in 10 of the 12 samples ($P<0.007$).[16] Since antigen effects on thymocyte selection are dose-dependent,[19] this increased *INS* expression from VNTR class III chromosomes (ie, gain of function) might enhance thymic tolerance to insulin, thus explaining the dominant protective effect of this polymorphism. This is in favor of insulin being the *IDDM2* gene, but does not rule out the possibility that *IGF2* is involved, either in addition to *INS* or in place of *INS*.

IGF2 AS A CANDIDATE GENE FOR *IDDM2*

The reasons to consider the product of *IGF2* in the pathogenesis of type 1 diabetes are summarized in Table 2. It is well appreciated that the IGF axis is important in lymphopoiesis and immune function.[20,21] IGF-2 is synthesized in and has biologic effects on tissues that are important in the pathophysiology of diabetes, such as pancreas, thymus, and lymphocytes. It is a particularly important fetal mitogen that has been shown to stimulate proliferation and/or prevent apoptosis. It has been hypothesized that IGF-2 may either function as a tolerogenic autoantigen in the thymus[22] or promote survival of self-reactive lymphocytes.[20] Involvement of IGF-2 in the pathogenesis of type 1 diabetes could explain parental imprinting effects reported in the genetics of *IDDM2*. Imprinting refers to differential transcriptional behavior (typically silencing) of a gene copy on the basis of the sex of the parent from whom it was inherited,[23] such as has been demonstrated for *IGF2* in some

Table 2
Arguments Supporting a Role for *IGF2* in Type 1 Diabetes Pathophysiology

Physiologic Arguments*

1. IGF-2 and type I IGF-1 receptor (mitogenic receptor) expressed in human fetal pancreas, thymus, and leukocytes
2. Type 2 IGF-2 receptor (involved in IGF-2 clearance) highly expressed in fetal thymus; suggests modulating IGF-2 levels may be important
3. IGF-2 transgenic animals show changes in lymphoid tissue, including clonal expansion of thymocytes, mainly mature CD4+ cells
4. IGF-2 known to promote cell survival (anti-apoptosis factor)

Genetic Arguments†

1. *INS* and *IGF2* mapped to within 2 kb of each other, a distance compatible with shared enhancer effects
2. Type 1 diabetes susceptibility in offspring of diabetic parents is related to paternal transmission (in some populations), suggesting imprinting effects
3. *INS* is not imprinted in human fetal pancreas; *INS* also is biallelically expressed in the majority of fetal thymi
4. *IGF2* is imprinted and expressed from the paternal allele in fetal pancreas and thymus
5. Placental *IGF2* mRNA levels are correlated with VNTR class
6. The 5' *INS* VNTR has transcriptional effects on *IGF2* (artificial constructs)

*See Polychronakos et al[20] for literature review
†References given in text.

tissues in the human fetus and in the human placenta.[24] In contrast, *INS* is not imprinted in human fetal or adult pancreas,[12,20,25] despite the imprinted expression of the mouse insulin genes, *Ins1* and *Ins2,* in yolk sack, where only the paternal copies are transcribed.[26]

Linkage[27] or association at *IDDM2* has been reported in alleles of paternal[28-30] or maternal[12,25] origin, but other studies have found no parent-of-origin effect.[31] In addition to the intriguing mechanistic questions they raise, these parent-of-origin effects may provide clues as to the genes and biologic mechanisms involved at the *IDDM2* locus. Paternal bias would favor *IGF2* because of its exclusive paternal expression; an explanation for the maternal bias is less obvious, unless one postulates longer-range VNTR effects on imprinted, maternally expressed genes at the 11p15.5 locus.

We have recently demonstrated that higher steady-state *IGF2* mRNA levels are associated with paternal class I VNTR alleles in normal human placenta, a tissue in which *IGF2* is exclusively expressed from the paternal allele. Furthermore, by using a construct in which a class I or class III VNTR is placed upstream of an important fetal *IGF2* promoter (P3), greater reporter gene activity is observed with the class I VNTR allele. The physiologic significance of this could relate to the anti-apoptotic effect of IGF-2 on self-reactive T cells during fetal life, whereby the class I VNTR alleles, via increased *IGF2* transcription, favor the development of diabetes because of survival of self-reactive lymphocytes.[32]

It should be stressed that dividing VNTR alleles into classes affords only a broad categorization, as each class contains many distinct alleles of varying sizes. It is possible that expression of *IGF2* and/or *INS* associated with particular VNTR alleles also is affected differently by parental imprinting, known to be a tissue-specific, developmentally-specific, and polymorphic phenomenon.[23] In the case of *INS*, the higher thymic transcript levels from the class III haplotype seen in 10/12 specimens is present independently of the parental origin of the class III. However, imprinting is likely involved in the complete silencing of the class III allele in the remaining 2/12 thymi.[16] Pugliese et al[17] also found monoallelic *INS* expression in 3/10 thymi. In all 5 cases, it was the class III haplotype that was silenced. Therefore, it is possible that imprinting of thymic *INS* in this minority of individuals requires the presence of *specific* class III alleles. A precedent for such haplotype-restricted imprinting has been described: the polymorphic silencing[33] of the paternal copy of *IGF2R* (the IGF-2 receptor gene) is controlled by a sequence variant in cis.[34] Imprinted *IGF2* expression might be modulated by a similar phenomenon in leukocytes, where there is variable "relaxation" of imprinting, thereby allowing transcription from the maternal copy to a variable extent in different individuals.[15,20] Dependence of relaxation on specific VNTR alleles could explain the maternal effect, if *IGF2* were involved in the *IDDM2* effect instead of (or in addition to) *INS*.

CONCLUSIONS AND FUTURE DIRECTIONS

Genetic evidence and functional considerations point to *IGF2* and/or *INS* as the *IDDM2* gene(s). Our knowledge of how *IDDM1* and *IDDM2* contribute to the diabetes phenotype continues to increase and is far in advance of our understanding of the many additional loci recently implicated. Furthermore, while the specific genes involved at these other loci are unknown at present, it is of interest that several genes coding for products that interact with IGF-2 can be found within some of them, such as those coding for the type 1 (15q26) and type 2 (6q27) IGF receptors and the IGF-binding proteins (2q34, 7p13). Is this mere chance, or does it explain some of the epistatic genetic interactions, whereby these loci are not independent but may be acting on the same or overlapping pathways involved in the pathophysiology of diabetes? With the speed at which technologic advances have accelerated our understanding of diabetes in the past 5 years, one can hope to see these questions answered in the not too distant future.

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Congenital Leptin Deficiency Is Associated With Severe Early-Onset Obesity in Humans

The investigators report that 2 consanguineous, first-cousin offspring with hyperphagia and morbid obesity beginning in infancy had a homozygous abnormality of the *OB* gene encoding leptin, the appetite-regulating protein secreted by the fat cell. Each child had a homozygous deletion of 1 guanine nucleotide in codon 133 leading to a frameshift mutation and 14 altered amino acids. This truncated leptin protein could be synthesized but not secreted from Chinese hamster ovary (CHO) cells transfected with the mutant gene, and both children had extremely low serum immunoreactive leptin levels. They were hyperinsulinemic and normocortisolemic. The parents of both children and 1 sibling were heterozygous for this mutation; their serum leptin levels were normal, as was their body fat content.

Montague CT, et al. *Nature* 1997;387:903-908.

Editor's comment: *These observations directly demonstrate for the first time the importance of leptin in appetite regulation in humans. All the obese subjects studied previously have not had an abnormality of genes encoding leptin or its receptor. The data also reveal that the heterozygote with 1 abnormal* OB *is normal. The phenotype of these children with hyperphagia, obesity, and hyperinsulinemia was quite similar to that of the* ob/ob *mouse. It differed from the animal model in that the linear growth of the children was normal (75th percentile for chronologic age; no bone age data given), and they were not hypercortisolemic.*

Jackson et al described a patient with childhood-onset obesity with dual mutations in the gene encoding prohormone convertase 1 (PC1), an endopeptidase necessary for prohormone processing. The patient was a compound heterozygote. One PC1 gene contained a Gly483Arg mutation that caused trapping of the gene product within the endoplasmic reticulum. The other PC1 gene had an A→C transversion in the donor splice site of exon 5, leading to deletion of this exon and a frameshift that resulted in a premature stop codon and truncated PC1 protein. The investigators hypothesized that loss of PC1 activity led to impaired processing of many protein prohormones, including neuropeptides involved in appetite regulation such as α-melanocyte-stimulating hormone and glucagon-like peptide 1.

We truly are on the threshold of understanding the relationship between leptin, genetics, and obesity.

Allen W. Root, MD

Jackson RS, et al. *Nat Genet* 1997;16:303-306.

Relationship Between Serum Leptin Concentration and Fetal Growth

Two recent articles in the *Journal of Clinical Endocrinology and Metabolism* concerned the relationship between the concentration of serum leptin and fetal growth.

Harigaya et al elucidated the role of leptin in the fetus. Blood samples from 116 infants were analyzed within 6 hours after birth. There was no difference in the concentration of leptin found in umbilical cord sera and infants' sera obtained within that 6-hour period. Ninety-one of these infants were term; 44 were classified as AGA (birth weight appropriate for gestational age); 28 were LGA (birth weight large for gestational age); and 19 were SGA (birth weight small for gestational age). Twenty-five were preterm. Infants with dysmorphic features, intrauterine infections, organic disorders, or chromosomal anomalies were excluded. Blood samples were compared with 28 umbilical cord samples taken at birth from the term group and 25 samples from healthy adults. Serum concentration of leptin and insulin levels were determined by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), respectively. Follow-up samples were taken from 48 of the 116 infants between 2 and 7 days of life. Leptin levels in term AGA infants were significantly lower than those of normal adults. The serum leptin concentration in LGA infants was significantly higher (12.8 ± 10.2 ng/mL) and those in SGA infants (1.6 ± 1.1 ng/mL) was significantly lower than in AGA infants ($P<0.01$). The follow-up leptin concentration in 48 term infants in the LGA and AGA groups dramatically decreased within 48 hours of delivery; the leptin concentration did not change in the SGA group. A positive correlation was found between leptin concentrations within 6 hours of life and birth weights ($r=0.59$, $P<0.01$). Leptin levels within 6 hours of life positively correlated with gestational age. The authors concluded that serum levels of leptin correlate with the fetal body weight gain.

Koistinen and colleagues looked at leptin concentrations in cord blood to see if there was a correlation with intrauterine growth. To determine how fetal growth compares with leptin levels at birth, 50 full-term infants were studied (28 = AGA; 9 = LGA; and 13 = SGA). Blood samples to measure leptin and insulin levels were taken from umbilical cord at birth. Amniotic fluid samples were obtained by amniocentesis from 10 mothers within 1 to 8 days before delivery and from 20 mothers at the time of Cesarean section. Umbilical leptin levels were higher in LGA infants (35.7 ± 8.0 µg/L; $P<0.005$) but lower in the SGA infants (3.3 ± 0.05 µg/L; $P<0.001$) than in AGA infants (14.5 ± 2.8 µg/L; $P<0.005$). Cord leptin levels correlated with birth weights, cord insulin concentrations, placental weight, and amniotic fluid leptin concentrations. Leptin concentrations in amniotic fluid were higher in LGA infants

than in AGA infants (4.8 ± 0.7 µg/L vs 3.1 ± 0.5 µg/L; $P<0.03$). The authors concluded that the strong relation between body weight and leptin concentration at term suggests that fatty mass is a major determinant of leptin secretion in utero.

Harigaya A, et al. *J Clin Endocrinol Metab* 1997;82:3281-3284.
Koistinen HA, et al. *J Clin Endocrinol Metabl* 1997;82:3328-3330.

Editor's comment: *Although the physiologic role of leptin levels in utero is not completely understood, these 2 papers report new data on leptin levels at birth, their correlation with fat mass, and their postnatal decline during the first week of life.*

The strong positive correlations found between serum leptin level and body weight gain in utero underscore the importance of this peptide as a marker of fetal growth. Thus, leptin could be useful as a predictive factor of fetal outcome, although further studies need to be done to ascertain this fact. Insulin and leptin levels do not correlate significantly in Harigaya's study, suggesting different mechanisms of fetal growth modulation by these 2 growth factors in utero.

Of interest is that both groups used the same assay but did not get the same results for AGA infants. In Koistinen's paper, the figure of 14.5 ± 2.8 µg/L was given, but in Harigaya's paper the value was 4.4 ± 3.0 µg/L. The reason for this discrepancy is not apparent.

These papers supplement the lead article on leptin by Zhang and Leibel in this issue of GGH *as Zhang and Leibel did not have the opportunity to present data on intrauterine growth and leptin levels.*

Fima Lifshitz, MD

Growth, Genetics, and Cancer

There is an undeniable relationship between cancer, growth, and genetics. Paraphrasing Eric R. Fearon, cancer is a genetic disease that arises from the accumulation of mutations that promote selection of clones of cells that display increasingly aggressive growth characteristics. Much of what is known about cancer genetics has come from studying hereditary cancer syndromes. Even though they collectively represent only about 1% of cancers, they have provided much insight into the pathogenetic mechanisms that give rise to cancer.

Fearon has recently examined 22 different hereditary cancer syndromes from a gene product functional perspective. Moreover, he has done this in the context of key cellular processes, such as cell proliferation, differentiation, apoptosis, and maintenance of genomic integrity. Thus, he organizes the syndromes into several functional categories. For example, several of the proteins are transmembrane receptors (proteins encoded by *MET, PTCH, RET*). Others are cytoplasmic regulatory or structural proteins (proteins encoded by *NF1, PTEN, APC, NF2*), transcription factors or regulators (proteins encoded by *p53, WT1, RB1, VHL*), or cell cycle regulators (proteins encoded by *CDK4, p16*). Finally, many proteins are involved in repair of DNA damage (proteins encoded by *MSH2, MLH1, PMS2, ATM, BRCA1, BRCA2, FACC, FACA, XPA, XPB, XPD, BLM*).

Several interesting observations come from this analysis. For instance, when genetic heterogeneity has been found, ie, hereditary nonpolyposis colorectal cancer, inherited melanoma, and familial breast cancer, all of the implicated genes function in a conserved pathway. For example, *MSH2, MLH1*, and *PMS2* in patients with hereditary nonpolyposis colorectal cancer adversely affect DNA mismatch recognition and repair.

One of the puzzling observations is that cancers are limited to certain tissues in most syndromes, yet the genes are widely expressed. It is suggested that many of the implicated genes function in interesting or overlapping pathways that branch and converge differently in different cell types. Another explanation is that genes simply may have different functions in different cell types. Fearon emphasizes that other factors, such as other genes, diet, environment, and lifestyle, substantially affect the expression of cancer in mutation carriers.

Fearon ER. *Science* 1997;278:1043-1050.

Editor's comment: *This excellent review puts a different slant on hereditary cancer syndromes. It not only organizes information from 10 years of literature concerning cancer syndromes but also presents the material in a functional context that allows one to create a big picture of how the syndromes relate to one another and to normal biologic processes.*

William A. Horton, MD

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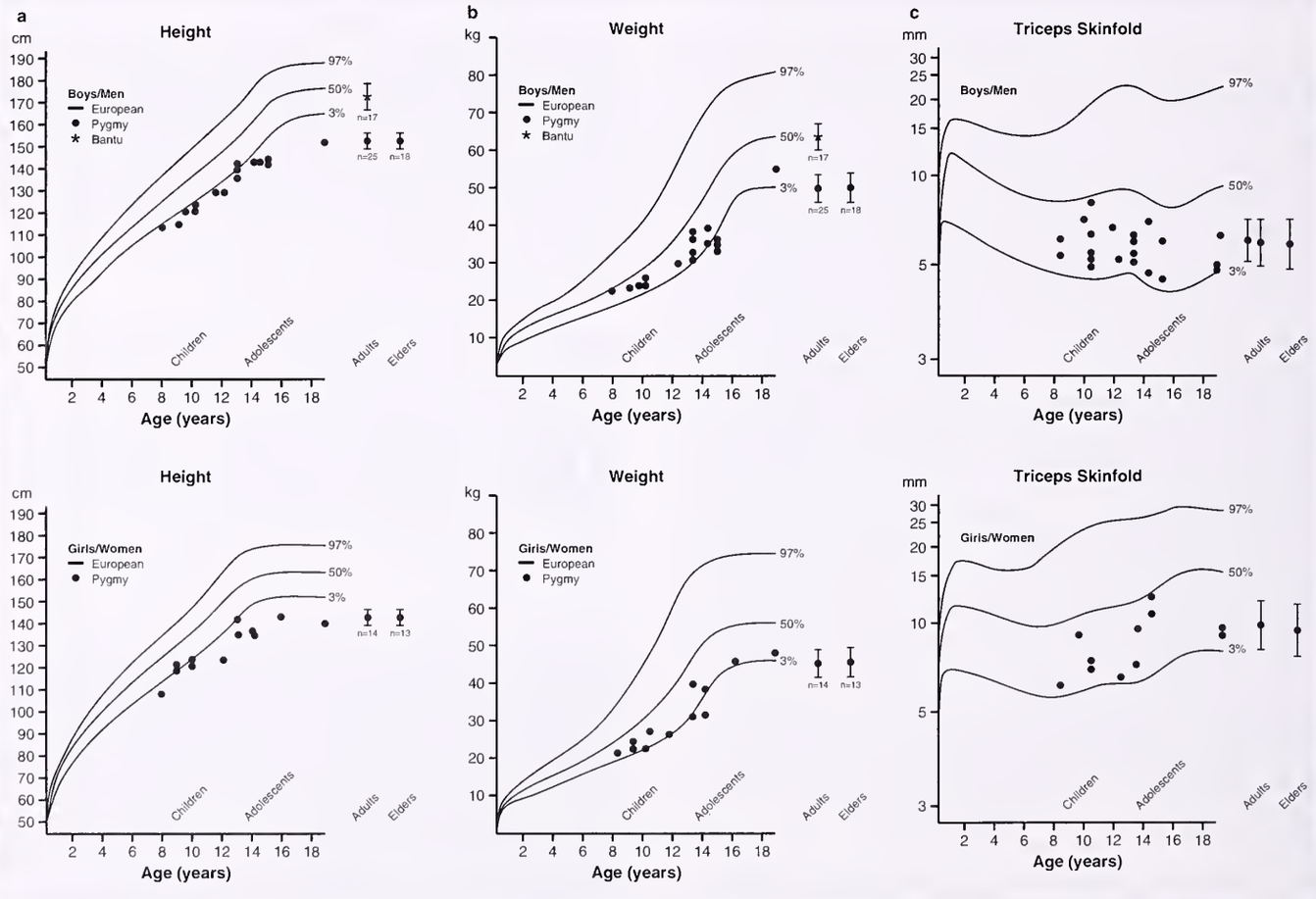
Dissociation of Systemic GH–IGF-1 Axis From a Genetic Basis for Short Stature in African Pygmies

The investigators tested the hypothesis that the primary cause of short stature in African Pygmies resides in low levels of insulin-like growth factor 1 (IGF-1) and evaluated whether any observed alterations in their systemic IGF-1 status could be associated with malnutrition and/or altered immune status. Extensive serum assays and auxologic measurements were done for purposes of evaluating the hormonal and immune status versus the phenotypes of children, adolescents, and young and old adults. None had overt clinical or biochemical signs of malnutrition. Bantus living in the same equatorial rain forest of eastern Cameroon were used as controls.

Pygmies did not differ from Europeans or Bantus in mean serum IGF-1 or IGF-binding protein 3 (IGFBP-3) levels. However, in both African groups, IgG, IgM, IgE, C-reactive protein, and ceruloplasmin were above normal for Europeans, but the Pygmies had much higher IgG and IgM levels than the Bantus. Low IGF-1 levels were inversely associated with serum levels of IgG and IgM.

The authors concluded that in growing and adult African Pygmies without evidence of clinical or biochemical signs of nutritional deficiency, serum IGF-1 and IGFBP-3 are essentially normal. They believe that these studies disprove the previous hypothesis that there is a defect in IGF-1 production or growth hormone insensitivity, and that the short stature of African Pygmies is unrelated to a genetically determined lesion that becomes unmasked at puberty to suppress the surge in circulatory IGF-1 levels. They suggest that much of the



Height (panel a), weight (panel b), and triceps skinfold thickness (panel c) plotted against age for African Pygmies are shown with a reference European (British) population (Tanner et al, 1966 a,b; Tanner & Whitehouse, 1975). Data for boys/men and for girls/women are shown on top and bottom part of the figure, respectively. For each anthropometic measurement, individual values are shown for children and adolescents, while mean and SD are shown for adults and elders; in addition the mean and SD values for the Bantu men are indicated using an asterisk symbol. Note that the heights of all Pygmies are below or close to the 3rd percentile of the European standard.

Reprinted with permission from Dulloo AG, et al. Dissociation of systemic GH–IGF-I axis from a genetic basis for short stature in African Pygmies. *Eur J Clin Nutr* 1996;50(6):371-380.

growth retardation of Pygmies may be due to excessive exposure to infections with resultant elevation of immunoglobulins, in spite of absent gross nutritional deficiency secondary to infection.

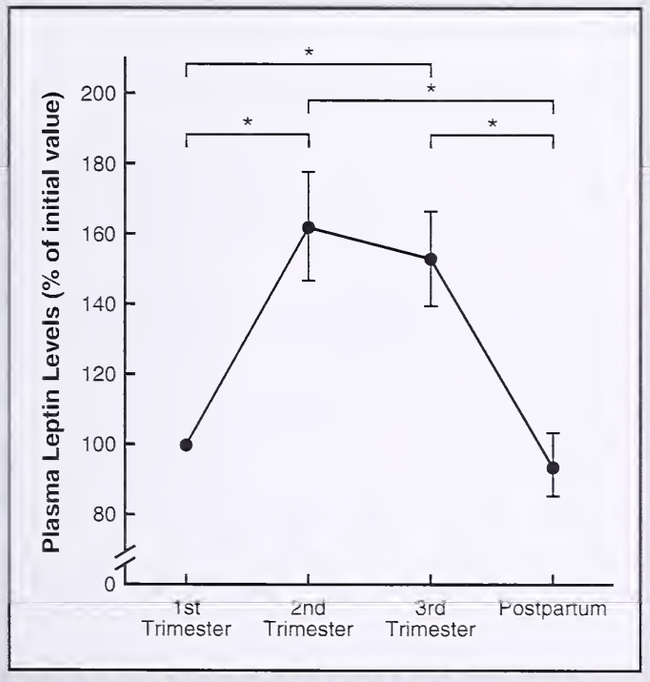
Dulloo AG, et al. *Eur J Clin Nutr* 1996;50(6):371-380.

Editor's comment: *This study is very nicely executed, and extensive data are presented to raise much skepticism about the previous hypothesis that an increase in IGF-1, which is normally associated with the adolescent growth spurt, does not occur in Pygmies. The data not only negate the former hypotheses but also reveal a new biochemical alteration (elevated immunoglobulins) that, in my opinion, may or may not be associated with growth retardation. This article was kindly brought to my attention by Dr. Guy Van Vliet.*

Robert M. Blizzard, MD

Nonadipose Tissue Production of Leptin: Leptin as a Novel Placenta-Derived Hormone in Humans

The authors report that leptin, the appetite-regulating polypeptide hormone secreted by adipocytes, also is synthesized and secreted by human placental chorionic villi and amnion cells. They demonstrated that plasma leptin concentrations were higher in pregnant women than in nonpregnant women of comparable body mass index (BMI), a finding made by other investigators. Whereas in nonpregnant women plasma leptin concentrations and BMI were directly related, this relationship was not demonstrable in pregnant subjects. Leptin values declined rapidly after birth. Umbilical vein leptin concentrations were slightly higher than umbilical artery values. Leptin was detectable in amniotic fluid. Plasma leptin values were elevated in patients with hydatidiform moles and choriocarcinoma and fell rapidly with surgical removal or chemotherapeutic ablation of the tumor.



Elevated plasma leptin levels during pregnancy. Time course of plasma leptin levels determined consecutively during pregnancy and postpartum (n=40). The plasma leptin levels in the first trimester are defined as 100%. * P<0.005 versus the values in the first trimester or postpartum.

Reprinted with permission from Masuzaki H, et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med* 1997;3:1029-1033.

Expression of *ob*, the leptin gene, was demonstrated in placental chorionic villi and amnion. Immunoreactive leptin was present in trophoblasts of first trimester chorionic villi and syncytiotrophoblasts and amnion cells in the third trimester. The investigators suggest that placental leptin may be of physiologic importance in modulating the metabolic relationship between mother and fetus. Plasma leptin values may serve as a marker of tumors of placental origin.

Masuzaki H, et al. *Nat Med* 1997;3:1029-1033.

Editor's comment: *This paper reports that leptin may be secreted by nonadipose placental cells, namely, placental trophoblasts and syncytiotrophoblasts, and secreted into amniotic fluid and the maternal circulation. Thus, leptin is another of the many protein hormones that are secreted not only by their primary tissue but also by the placenta. Placental leptin may modulate metabolic homeostasis in the pregnant woman and fetus. One wonders if, in the pregnancy complicated by placental insufficiency and intrauterine growth retardation, leptin deficiency may permanently alter hypothalamic appetite regulatory mechanisms that influence the postnatal growth of that individual.*

Chehab points out that the elevated leptin concentrations in pregnant women imply an element of leptin resistance because appetite is not suppressed by pregnancy. Although these investigators did not note correlation between BMI and serum leptin concentrations in pregnant women, Hartmann et al reported that the 2 were highly correlated.

Allen W. Root, MD

Chehab FF. *Nat Med* 1997;3:952-953.
Hartmann BW, et al. *N Engl J Med* 1997;337:863.

A Dominant-Negative Mutation of the Growth Hormone Receptor Causes Familial Short Stature

Ayling and colleagues report the identification of a new dominant-negative mutation in the growth hormone receptor (GHR) in a mother and daughter with short stature. This mutation (876-1 G→C transversion affecting the 3' splice acceptor site preceding exon 9) was not detected in maternal grandparents. The mutation results in a premature stop codon or a truncated GHR. The GHR belongs to a cytokine family of receptors that depend on JAK tyrosine kinases for activation. It is predicted that a truncated receptor would be incapable of association with JAK and, therefore, would have a dominant-negative effect on the GHR.

The clinical significance of this mutation relates to the phenotypic differences of the individuals from those with the Laron syndrome, in which midfacial hypoplasia, blue sclera, limited elbow extension, hypoglycemia, truncal adiposity, etc are often observed. In the latter, the mutations of the GHR have been primarily in the extracellular domain. In the 2 patients reported here, the GHBP, the extracellular portion of the GH binding receptor, was normal. The authors suggest that this new dominant GHR mutation should be looked for in children with familial short stature who have normal GHBP (as opposed to low GHBP, which triggers the search for GHD genetic abnormalities currently), a group of children who were previously felt to have no known endocrine cause of their short stature.

Ayling RM, et al. *Nat Genet* 1997;16:13-14.

***Editor's comment**: This is an exciting contribution to the rapidly growing fund of information regarding the molecular causes of growth failure in children. It is not uncommon for a pediatric endocrinologist to be faced with extremely short children for whom no endocrinopathy can be identified. The work by Ayling and colleagues describes an additional genetic mutation that could present as growth hormone insensitivity syndrome. We look forward to studies of other families in hopes of determining the clinical magnitude of this new finding. The implications for potential treatment with insulin-like growth factor (IGF-1) are obvious, although the availability of IGF-1 as a therapeutic agent seems far distant in the future.*

William L. Clarke, MD

Androgen Insensitivity With Mental Retardation: A Contiguous Gene Syndrome?

A contiguous gene syndrome is the combination of clinical features resulting from a microdeletion of chromosomal DNA involving 2 or more contiguous gene loci. The location for the androgen receptor gene is Xq11-q12. Davies et al have identified a syndrome involving mental retardation and androgen insensitivity at Xq11.2-q12 between DXS1 and DXS905. Two affected individuals are reported with complete androgen insensitivity. This suggests that a gene for nonspecific mental retardation lies very close to the androgen receptor gene. They analyzed 4 patients with androgen insensitivity, 2 of whom also had mental retardation. Deletion analysis of the 2 individuals with mental retardation showed that the deletion extended past the androgen receptor gene in both directions, whereas in the individuals without mental retardation, the deletion was limited to the androgen receptor gene itself.

Androgens (testosterone and dihydroepiandrosterone) are steroid hormones secreted by the adrenal cortex that promote male sexual differentiation. The androgenic effect is mediated by the intracellular androgen receptors. Certain androgens bind to the androgen receptor and cause masculinization of the developing male fetus. A defect in the androgen receptor gene results in androgen insensitivity, which is a disorder of male sexual differentiation. This occurs when the target tissues fail to respond to the male sex hormones (androgens), ie, the receptors are insensitive or resistant because of a defect in the androgen receptors (mutations). Androgen insensitivity syndrome can be complete or partial. In complete

androgen insensitivity, the individuals have a normal male karyotype (46,XY), testes, and external genitalia like those of females (female phenotype). In partial androgen insensitivity syndrome, the receptor affinity is decreased for the ligand. The phenotype is extremely variable.

Davies HR, et al. *J Med Genet* 1997;34:158-160.

Editor's comment: *Deletion analysis of the androgen receptor gene has improved our understanding of the causes of androgen insensitivity. More than 150 point mutations have been reported so far. The phenotype is extremely variable, probably reflecting the heterogeneity of the androgen receptor gene mutations. One cannot predict the phenotype based on these mutations because the same mutation may be associated with a different phenotype, suggesting that other modifying genes play a role in androgen response. Mutations in the androgen receptor gene have been described in a number of clinical situations, including male infertility, prostate cancer, breast cancer, and Kennedy's disease. It is of particular interest that patients with androgen insensitivity and mental retardation have large deletions. There are many genes for mental retardation on the X chromosome; however, these 2 patients suggest at least 1 mental retardation gene is very close to the androgen receptor. Mental retardation seen in the other clinical situations involving the androgen receptor gene also may suggest contiguous gene deletions.*

Judith G. Hall, MD

Growth Pattern During the First 36 Months of Life in Congenital Adrenal Hyperplasia (21-Hydroxylase Deficiency)

Gasparini et al followed 17 female and 7 male infants with congenital adrenal hyperplasia due to 21-hydroxylase deficiency from diagnosis until 36 months of age. All were initially treated with cortisone acetate (25 mg/m^2/d in 3 divided doses given q8h) and 9α-fluorohydrocortisone (0.05 mg q12h). Every 3 months for the first 12 months and every 6 months thereafter, the height was recorded as length for chronologic age (CA) and ponderal growth as percentage of ideal body weight (IBW). Annual bone ages (BA) were obtained. At diagnosis, males tended to be less compromised in linear growth than females (standard deviation score for less [SDS-L] -0.5 ± 0.7 vs -1.1 ± 1.1) but were significantly more compromised in weight (76.3 ± 16.7% IBW vs 91.7 ± 8.0%, P<0.05). By 3 months of age, the females' percentage of IBW remained unchanged; that of males normalized and remained similar to females through the next 36 months of observation. SDS-L at 3 months in females increased to 0.41 ± 0.88 (P<0.005) and remained constant thereafter. Males, on the other hand, showed a significant and progressive decrease in SDS-L at 6 months (-1.41 ± 0.96; P<0.05), but from that point on showed a progressive increase, reaching and maintaining normal values by 18 to 24 months of age. No differences were noted between males and females with regard to 17-hydroxyprogesterone (17-OHP) levels or the BA:CA ratio, which approximated 1. 17-OHP levels were distributed over a wide range, and in all patients a correlation was found between SDS-L for target height and the SDS-L at 2 years and 3 years. In particular, the SDS-Ls of both males and females at 3 years were comparable to that of the midparental height of their parents.

The authors point out that the traditional treatment of 21-hydroxylase deficiency has included cortisone acetate, 25 mg/m^2/d. However, the decrease observed in linear growth in the males was interpreted as possible overtreatment; thus, the cortisone acetate dose was decreased despite elevated levels of 17-OHP. Twelve of the patients were followed longitudinally until 7 years of age. Those individuals maintained a BA:CA ratio between 0.83 ± 0.19 and 1.01 ± 0.29 despite cortisone acetate doses between 15.9 ± 6.0 mg/m^2/d and 20.0 ± 8.0 mg/m^2/d. These patients reached a height that correlated with their predicted adult height despite inadequately suppressed 17-OHP, at least for the first 7 years of life.

Gasparini N, et al. *Horm Res* 1997;47:17-22.

Editor's comment: *This is an important and interesting paper. It should provide encouragement to those who utilize cortisone acetate replacement therapy at doses of <25 mg/m^2/d in attempts to maintain normal linear growth in their patients despite elevated 17-OHP levels. The reader is referred to a recent review (*J Clin Endocrinol Metab *1996;81:3180-3191) on the use of adrenalectomy as treatment for congenital adrenal hyperplasia and for further discussion of a variety of*

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different treatment options available for patients with this disorder. Unfortunately, in the United States, the major supplier of injectable cortisone acetate has discontinued its production, and pediatric endocrinologists will be forced to become familiar with other glucocorticoid agents or other forms of treatment.

Gasparini et al did not report on any adverse clinical events during times of physiologic stress that may have occurred in patients receiving <25 mg/m²/d. Such information would be important to have prior to making recommendations regarding their proposed therapeutic regimen.

William L. Clarke, MD

2nd Editor's comment: *The reader also is referred to an article by Kerrigan et al (*J Clin Endocrinol Metab *1993;76:1505-1510), reporting that the production rate of hydrocortisone is less than that calculated by Migeon's group (approximately 6.1 mg/m²/d vs approximately 12 mg/m²/d). It is not surprising, therefore, that a dose less than twice the production rate (25 mg/m²/d), which is the dose previously accepted by endocrinologists as being a suppressive dose in congenital adrenal hyperplasia, may be more than necessary to adequately treat the disorder.*

Robert M. Blizzard, MD

Opitz Syndrome Gene Found

Optiz syndrome, which was originally described as G and BBB syndromes, is characterized by midline defects, including hypertelorism, hypospadias, lip/palate/laryngotracheal clefts, and imperforate anus. Clinically indistinguishable forms have been genetically mapped to the X (Xp22) and 22 (22q11.2) chromosomes. A consortium of several groups (see references) headed by Andrea Ballabio in Milan has now found the gene that harbors the mutations responsible for the X-linked form.

After first constructing a physical map of the Xp22 breakpoint region, the investigators next identified expressed sequences from which they were able to assemble a consensus cDNA sequence of 3,452 bp. The predicted protein product is 667 amino acids, which was named MID1. Expression of the *MID1* gene was then studied. Transcripts were found in virtually all normal fetal tissues examined, especially kidney, brain, lung, and placenta, and in the heart and brain of adults. No transcripts were detected in samples from an affected male. Studies in the mouse revealed that *mid1* is expressed ubiquitously in early embryos, with the highest levels found in the first and second branchial arches.

The sequence of predicted protein places it in the so-called B-box family of zinc-finger proteins. These proteins contain a "RING-finger" and 2 "B-box" domains, which are thought to mediate protein-protein interactions. Genes belonging to this family encode transcriptional regulators.

Mutation analyses were carried out in patients from 22 independent families; mutations were found in 4 families. The gene was disrupted by the pericentric inversion in the family used for mapping. The other mutations were an in-frame 3-bp deletion, a frameshift that produces a premature stop codon, and a tandem duplication of 24 bp. All are predicted to disrupt the function of the protein.

The authors conclude that the *MID1* encodes a protein whose function is important for development of midline structures.

Green EA, et al. *Science* 1997;278:615-630.
Henikoff S, et al. *Science* 1997;278:609-614.
Quaderi NA, et al. *Nature Genet* 1997;17:285-291.
Tatusov RL, et al. *Science* 1997;278:631-637.

Editor's comment: *Genes relevant to early human development are being discovered at a rapid pace, primarily from positional cloning of "birth defect syndrome" genes. This is very exciting, but it is difficult to keep track of the different genes and mutations. The situation is analogous to the chondrodysplasia situation in the early 1990s, when there were well over 100 distinct disorders, multiple ways of classifying them, and considerable debate over grouping versus separating conditions with subtly different clinical features. Fortunately, molecular genetics helped to sort out the situation by revealing that a large portion of these disorders fell into a relatively small number of "chondrodysplasia families" that shared common genetic origins.*

The situation is more complicated when the entire spectrum of birth defects is considered. However, attempts are now being made to "organize" genes into families, which will likely lead to a reconsideration of how these syndromes are classified and managed nosologically. This move to better organize genetic information into a family context was very evident at the 1997 American Society of Human Genetics meetings in Baltimore, as well as in the 1997 Genomics issue of Science. *These efforts should eventually help keep clinicians from becoming lost in the maze of molecular genetic information.*

William A. Horton, MD

In The Next Issue

Growth Hormone Replacement in Adults
Peter Sönksen, MD,
and J Weissberger, MD

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2. a b c d
3. a b c
4. a b c
5. a b c
6. a b c d
7. a b
8. a b c d
9. a b c d
10. a b c d

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5 = Excellent 4 = Above average 3 = Good 2 = Below average 1 = Poor

Please evaluate this course with respect to the following:

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4. Length of articles	5	4	3	2	1
5. Were the educational objectives met?	5	4	3	2	1

6. In your opinion, how could this newsletter be improved?

_____

_____

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UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
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GROWTH, Genetics, & Hormones

Volume 14, Number 2

Post-Program Self-Assessment/CME Verification
Answer Sheet

The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education activities for physicians.

The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Answer each of the questions or statements contained in the Post-Program Self-Assessment/Course Evaluation. There may be more than one correct answer. Participants are encouraged to refer to the related article for evaluation of their responses. To receive Category 1 credit, complete the self-assessment exam/course evaluation and record your responses on the answer sheet. Enclose a check for $20 made payable to the University of Virginia or complete credit card information. Mail the answer sheet and fee to:

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A certificate will be mailed to you. This enduring material was approved for credit June 1998. Eligibility for credit expires June 2001.

Evidence From Turner's Syndrome of an Imprinted X-Linked Locus Affecting Cognitive Function

The investigators studied 80 Turner syndrome (TS) patients with a single X chromosome. In 55 patients, the X was maternally derived (45,X^m) and in 25, paternally derived (45,X^p). Members of the 45,X^p group were significantly better adjusted, with superior verbal and higher order executive function skills, which mediate social interactions.

The investigators conclude that the observations suggest there is a genetic locus for social cognition that is imprinted by the X^p in TS patients and not by the X^m. Neuropsychological and molecular investigations of 8 females with only partial deletions of the short arm of the X chromosome indicated that the putative imprinted locus escapes X-inactivation, and probably lies on Xq or close to the centromere on Xp. If this locus is expressed only from the X chromosome of paternal origin, the existence of this locus could explain why 46,XY males, who always have a maternally derived X, are more vulnerable to developmental disorders of language and social cognition, such as autism, than are 46,XX females.

The techniques used in differentiating the behavioral differences of 45,X^p from 45,X^m are listed in the Table. The 2 groups, as compared with normal 46,XY and 46,XX, are presented in the Figure.

Skuse DH, et al. *Nature* 1997;387:705-708.

Editor's comment: *The hypothesis presented and the evaluation and testing of the hypothesis are unique and reflect the contributions of an important subgroup of investigators—specifically, the neuropsychoendocrinologists. Collaboration of neuropsychoendocrinologists with pediatric endocrinologists, geneticists, psychiatrists, and others is significantly contributing to therapeutic considerations with which endocrinologists and geneticists deal.*

The hypothesis of these investigators is not totally proven as there are other possible explanations for the findings, but the possible correctness of the hypothesis as demonstrated by the investigators will stimulate further and related studies that may expand our knowledge of the relationships between imprinting, growth, intellect, and social or cognitive function. Watch for further studies and developments.

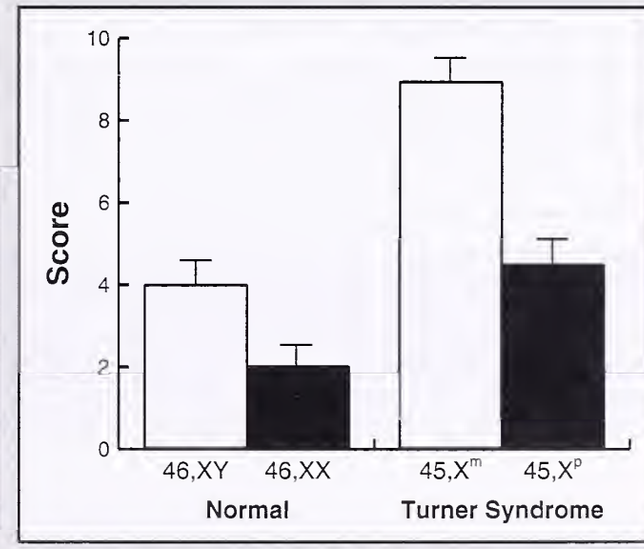
Robert M. Blizzard, MD

Scale for Measuring Social Cognition

Complete the following section by circling 0 if the statement is not at all true of your child, 1 if it is quite or sometimes true of your child, and 2 if it is very often true of your child:

0 1 2	• lacks an awareness of other people's feelings
0 1 2	• does not realize when others are upset or angry
0 1 2	• is oblivious to the effect of his/her behavior on other members of the family
0 1 2	• behavior often disrupts normal family life
0 1 2	• is very demanding of people's time
0 1 2	• is difficult to reason with when upset
0 1 2	• does not seem to understand social skills, eg, interrupts conversation
0 1 2	• does not pick up on body language
0 1 2	• is unaware of acceptable social behavior
0 1 2	• unknowingly offends people with behavior
0 1 2	• does not respond to commands
0 1 2	• has difficulty following commands unless they are carefully worded

Internal consistency for set of 12 questions: Standardized item alpha 0.94



Subscale scores (mean + SE) of questionnaire on social-cognitive impairment (see Table). Higher scores indicate poorer social cognitive skills. The 45,X^m Turner syndrome females score higher than 45,X^p females and both normal groups ($P<0.0001$). Normal males score higher than normal females ($P<0.001$); the effect size of this difference is 0.58, implying that the upper 50% of females score higher than approximately 72% of males. The ratios of mean social-dysfunction scores male:female and 45,X^m:45,X^p are very similar (2.2:1 and 2.1:1, respectively). The overall higher scores for the Turner syndrome subjects, compared with normal females, may reflect the contribution made by visuospatial abilities to social cognition. These abilities are impaired equally in both monosomic groups. No information regarding parental origin of the normal X chromosome was made available to parents, their consultants, or members of the research team gathering these or other data.

Post-Program Self-Assessment/CME Verification

Instructions: The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on page 38 of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. The following characteristics occur in both *ob/ob* and *db/db* mice:
   a. obesity
   b. hypothalamic infertility
   c. impaired thermoregulatory thermogenesis
   d. large heads
2. Which of the following increase(s) expression of leptin mRNA?
   a. Thyroxine
   b. Cortisol
   c. Growth hormone
   d. Insulin
3. Leptin is virtually absent in the circulation of the _____.
   a. *ob/ob* mouse
   b. *db/db* mouse
   c. *fa/fa* rat
4. The two reported children with very low serum leptin concentrations differ from mice with leptin deficiency in the following respect(s):
   a. mild versus gross obesity
   b. normal versus high cortisol levels
   c. normal linear growth versus short linear growth
5. Central administration of neuropeptide Y _____.
   a. stimulates food intake
   b. stimulates sympathetic nervous activity
   c. stimulate plasma insulin
6. Familial clustering and a high concordance rate in monozygotic twins indicate that genetic transmission of susceptibility is responsible for about _____% of the risk for the development of insulin-dependent diabetes mellitus.
   a. 33%
   b. 50%
   c. 67%
   d. 75%
7. Reverse genetics is the determination of the chromosomal location and identification of new genes in spite of ignorance of the disease mechanism.
   a. True b. False
8. The application of reverse genetics to common polygenic phenotypes is _____.
   a. easy
   b. moderately easy
   c. moderately difficult
   d. difficult
9. _____ loci have been detected by genome-wide scans for susceptibility for insulin-dependent diabetes mellitus.
   a. Four b. Eight c. Thirteen d. Sixteen
10. Genetic evidence and functional considerations point to _____ as the *IDDM2* gene(s).
    a. *INS* b. *IGF2* c. *IGF1* d. *NPY*

Answer Key: 1. a,b,c 2. b,d 3. a 4. b,c 5. a,c 6. a 7. a 8. d 9. d 10. a,b

CME Accreditation Statement

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Drs. Deal, Polychronakos, Zhang, and Leibel report no conflicts. Drs. Lifshitz, Clarke, Horton, Hall, Rosenfeld, and Slyper report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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Growth Hormone Deficiency in Adults

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INTRODUCTION

Growth hormone deficiency (GHD) in adulthood (AGHD) has recently received much attention, particularly in Europe. There has been a dramatic upsurge of awareness of the role and importance of AGHD since a landmark epidemiologic study from Sweden showed an excess mortality in AGHD[1] and the results of the first double-blind, placebo-controlled trials of growth hormone (GH) replacement in AGHD showed material benefit.[2,3] Previously, it was widely assumed that GH had no physiologic relevance once linear growth ceased, and the limited availability of pituitary-derived human GH meant there was insufficient material to explore its effects in adults. The introduction of recombinant human GH (rhGH) in the mid-1980s and its subsequent use in clinical trials has forced a major reappraisal of the importance of maintaining an adequate presence of GH during adult life.

In the last 9 years, many European centers have reported their experience with rhGH in AGHD, mainly using formal placebo-controlled clinical trials. Many meetings have been held to discuss the results of these trials and their implications for routine patient care. Most of the patients entered into these trials had other pituitary hormone deficiencies, for which they received stable conventional hormone replacement in appropriate doses. Despite differences in study design, patient selection, and rhGH used, the findings have been remarkably consistent and unexpectedly positive. Collectively, they indicate that the majority of AGHD patients, whether the GHD is of childhood onset (COGHD) or acquired onset in adult (AOGHD) life, are compromised both physically and psychologically and can derive substantial, sometimes dramatic, benefit from GH replacement.

Many of the benefits that GH replacement brings are now well documented as a result of numerous double-blind, placebo-controlled trials.[4] Description of the new syndrome of AGHD has resulted,[5] the main features of which are presented in Table 1 (page 42) and are discussed in more detail below.

BODY COMPOSITION

Apart from stimulating longitudinal bone growth, GH is strongly anabolic and lipolytic and has a powerful antinatriuretic action. GHD children (CGHD) are not only short but also have abnormal body composition with excessive fat, reduced lean tissue, and contracted extracellular fluid (ECF). It is now clear that these abnormalities of body

In This Issue

Growth Hormone Deficiency in Adults.....page 41
Letter to the Editor.....page 42
Abstracts.....page 49
CME Information.....page 58

This educational activity is supported by an unrestricted educational grant from Genentech, Inc.

Letter to the Editor:

Recently, we evaluated a 40-year-old woman with McCune-Albright syndrome in whom breast cancer was diagnosed at age 38. At age 2 months, menarche and premature thelarche were diagnosed. Growth stopped at age 9. No therapy produced cessation of menses. The association of premature menarche and later development of breast cancer suggests a potential relationship between these 2 conditions. This patient was exposed to substantial estradiol from 3 months to 9 years of age. We postulate that this may have been sufficient to cause her breast cancer.

Known risk factors for breast cancer exist, including early menarche, late menopause, late pregnancy, prolonged use of hormone replacement therapy, obesity, and elevated plasma estradiol. The majority of these are associated with prolonged or increased exposure of breast tissue to estrogen. Early menarche and late menopause are obvious causes of increased duration of estrogen exposure. Obesity is associated with an increase in aromatase activity in adipose tissue and increased peripheral production of estrone and estradiol. Estrogen replacement during the menopause also increases exposure.

The most common hypothesis regarding estrogen-induced carcinogenesis is that estrogens bind to estrogen receptors; stimulate the transcription of estrogens, which enhance cellular proliferation; and increase the rate of proliferation. Increased estrogen exposure would be expected to cause an increased number of genetic mutations in breast tissue. The rate of proliferation also would decrease time available for DNA repair of new mutations.

An alternate hypothesis has been suggested. Estradiol can be metabolized to 4-OH-estradiol, a catechol estrogen, and then to 3,4-quinone. This compound can bind covalently to guanine, activate glycosidase, and result in depuration of DNA. Upon replication of cells with removal of guanine, the preferential substitution is thymidine during the replication process. This results in G → T point mutations.

A reasonable further hypothesis is that the genomic effect of estradiol to increase proliferation and the genotoxic effects of metabolites act in an additive or synergistic fashion to cause cancer. This concept was recently reviewed at a conference at the Westlands Center in Chantilly, Virginia, dedicated to discussion of the carcinogenic effects of estradiol.

We would like to determine if other patients with McCune-Albright syndrome also have experienced early onset of breast cancer. If a sufficient number of cases can be identified, the association would become more than anecdotal. We encourage physicians caring for adult McCune-Albright patients to respond to this letter by reporting other cases.

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Table 1
Clinical Features Suggesting Growth Hormone Deficiency in Adults

Past history:
- Known pituitary pathology/treatment
- Full conventional hormone replacement

Associated with symptoms (sometimes only on direct questioning), including:
- Impaired psychologic well-being
  - Poor general health
  - Reduced vitality and energy
  - Impaired emotional reaction
  - Depressed mood
  - Impaired self-control
  - Anxiety
  - Increased social isolation
- Increased abdominal adiposity
- Reduced strength and exercise capacity

Together with such signs as:
- Mixed truncal/generalized obesity
- Increased waist:hip ratio
- Thin, dry skin; cool peripheries; poor venous access
- Mild/moderate reduction in muscle strength
- Moderate reduction in exercise performance
- Psychologic state characterized by low, labile mood

Supplemented by test results:
- Stimulated growth hormone level <3 ng/mL
- Low or low-normal serum IGF-1
- Unfavorable serum lipid profile
- Low glomerular filtration rate and renal plasma flow
- Reduced lean body mass/increased fat mass
- Reduced basal metabolic rate
- Reduced bone density

composition also are present in AGHD[3,6] and are reversible with GH replacement.[2,3,7,8]

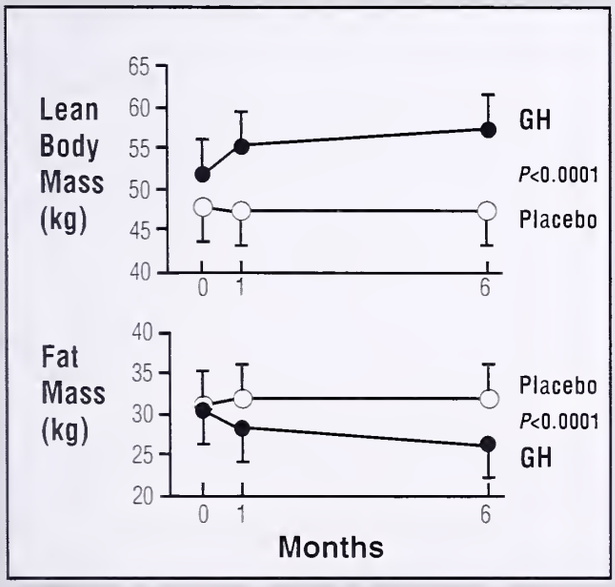
Fat Mass

In a cohort of 24 severely GHD adults that we first studied in 1987, mean fat mass (derived from the measurement of total body potassium) was 37.9% of body weight, exceeding that predicted from age, sex, height, and weight by an average of 7%.[3] This was very similar to the experience with a much larger cohort of 101 GHD Swedish adults who had a mean excess of body fat (determined from combined measurements of total body water and total body potassium) of approximately 6 kg.[6] As is the case in CGHD, the excess fat in AGHD tends to accumulate predominantly in the abdominal region and particularly in the intra-

abdominal organs.[8] A reduction in fat mass with GH replacement in adults has been a uniform finding.[2,3,8] In our patients, a mean reduction of 5.7 kg (18%) of body fat was observed after 6 months (Figure 1), with the greatest change occurring in the abdominal region, as shown by changes in skinfold thickness and waist:hip ratio. The selective effect of rhGH on abdominal fat in AGHD has been elegantly demonstrated by computed tomography (CT) scanning, with abdominal fat (both intra-abdominal and subcutaneous) found to decrease by an average of 30% after 6 months of treatment, compared with a 13% reduction in more peripheral sites.[8] These changes all occurred with no alterations in dietary intake. With calorie restriction, however, remarkable losses of fat have occurred with simultaneous increases in lean body mass (LBM). This is well demonstrated by the case of Mrs. E.L. (illustrated in Figure 2).

Mrs. E.L. developed pituitary apoplexy in 1953. Following this, she was given a course of deep X-ray therapy to prevent any tumor recurrence and given levothyroxine 0.15 mg, cortisone acetate 37.5 mg/d, DDAVP 0.1 mL/hs, and estrogen/progesterone. She remained on this regimen until 12 months before she was sent to St. Thomas', when her estrogen/progesterone preparation was withdrawn.

Figure 1



Changes in body composition (lean body mass and fat mass) during 6 months of GH replacement/placebo in 24 adults with longstanding GHD.

Adapted with permission from Salomon F, et al. *N Engl J Med* 1989;321:1797-1803.

Figure 2

Patient E.L.:

- GH commenced December 1990
- Maintenance dose: 0.04 - 0.05 U/kg/day
- IGF-1: 2.9 → 32.0 nmol/L

Patient E.L.— Body Composition

	Dec. '90	Dec. '91
Weight (kg)	104.7	78.0
Lean Body Mass (kg)	40.1	47.2
Fat Mass (kg)	64.6	30.8
Sum of Skinfolds (mm)	22.0	144.0

Case study of Mrs. E.L., who suffered from hypopituitarism secondary to pituitary apoplexy in 1953. She was wheelchair-bound and unable to lose weight until she started GH replacement in December 1990.

Over the preceding 5 years she had become increasingly weak. When seen at St. Thomas', she was virtually wheelchair-bound. Her weight had increased due to her inactivity and had resisted her continuous dietary attempts to control it. She was depressed, saw little future in life, and had begun to drink more alcohol than was good for her as a form of escape.

Following the addition of rhGH to her drug regimen, she lost weight dramatically (on the same calorie-restricted diet on which she had previously gained weight), while simultaneously substantially increasing her LBM. Her mobility increased dramatically, as did her quality of life. A subsequent long-overdue knee replacement went smoothly and improved her mobility further. She withstood additional corticosteroid and immunosuppressive therapy required for bullous pemphigoid, without significant loss of quality of life or mobility. She remains mobile and active, although her weight has crept up a little over the years.

Lean Body Mass

In our original study, GHD adults had values for LBM derived from total body potassium measurements that were 7% to 8% lower than values predicted from the age, sex, height, and weight of the subjects. After 6 months of GH replacement, LBM was normalized, increasing on average by 10% to 11% (5.5 kg), with most of this increase occurring in the first month of treatment (Figure 1). Other investigators, using several

different methods for determining LBM, have also demonstrated increases of 7% to 10% over a 6-month period of rhGH treatment. Open studies conducted over longer periods of rhGH replacement extending beyond 5 years suggest that the restoration of LBM is largely complete within the first 6 to 12 months, with less but still important increases occurring thereafter. This is in contrast to the usual loss in LBM with increasing age.

Skeletal muscle, which comprises approximately 50% of LBM, is substantially reduced in AGHD. The distribution of muscle and fat in cross-sectional mid-thigh CT scans from 22 adults with COGHD was 63% and 37%, respectively, compared with the distribution in healthy age-matched controls of 85% muscle and 15% fat.[2] We made similar observations in 20 patients with AOGHD, the mean cross-sectional area on CT of the dominant quadriceps muscle (expressed per kilogram body weight) being 15.5% lower than that in controls matched for age, sex, and activity. Following 6 months of rhGH treatment in AGHD, significant increases in cross-sectional muscle areas on CT of 5% to 8% have been observed in the thigh[2,9] and other regions.[8]

The deficit in LBM in untreated AGHD represents not only a loss of cell mass and tissue protein but also a substantial contraction of ECF. Estimates of extracellular water in 101 AGHD patients (based on the measurement of total body potassium and total body water) were on average 15% lower than predicted values.[6] After 6 months of rhGH treatment in 10 AGHD patients, there was a mean increase in LBM of 4.6 kg, of which as much as 3.0 kg could be attributed to increases in ECF.[8] These observations are in keeping with the well-described antinatriuretic action of GH[10,11] which appears to involve relatively minor activation of the renin-angiotensin-aldosterone axis[12-14] and a probably more important direct effect of rhGH and/or insulin-like growth factor 1 (IGF-1) on renal tubular sodium reabsorption.[15-17]

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Bone Mass

The importance of GH for skeletal growth and maturation in childhood is well established, but much less is known about its role in maintaining bone mass during adult life. Several studies have demonstrated reduced cortical and trabecular bone mass in AGHD of COGHD. In such cases, the osteopenia may be the result of a failure to achieve normal adult height and a deficient accretion of bone during childhood and adolescence rather than an accelerated loss of bone during adulthood. One study involving only patients with AOGHD, however, also demonstrated diminished cortical and trabecular bone mass.[17] Osteopenia was most marked in patients who had acquired GHD in early adulthood, again presumably because of a failure of accretion of sufficient bone in the years during which peak bone mass is normally attained. However, bone mass also was significantly reduced in those patients who had developed AOGHD after the age of 30 years, suggesting that GHD does indeed lead to a loss of bone after peak bone mass has been attained. This remains a slightly controversial topic. The balance of evidence indicates that GH is essential for achieving normal peak bone mass and that GHD with onset after peak bone mass has been achieved has a relatively minor, but still significant, effect on increasing the rate of bone loss. The clinical significance of the collective findings has not been firmly established, but there are at least 2 studies showing an increase in osteoporotic fractures in adults with GHD.[18,19]

We now know that GH replacement in adults stimulates the processes of bone remodeling, resulting in changes in various serum and urinary markers of bone turnover. In the early stages this may result in a decrease in bone density as remodeling is activated.[20,21] Only after about 6 months does bone density begin to increase with longer-term rhGH treatment.[21,22] Longer-term data over 5 or more years indicate that there is a steady gradual accumulation of bone; this continues in a linear fashion so long as the rhGH continues to be administered. After rhGH is stopped, the accretion of bone continues for at least 6 months.

Skin

The fine facial wrinkling characteristic of hypopituitary patients may be related to GHD. Skin thickness and total skin collagen are reduced in hypopituitary patients, despite conventional replacement therapy; the converse is true in acromegaly.[23] An increase in skin thickness was demonstrated following rhGH treatment in normal elderly males selected on the basis of low IGF-1 levels.[24]

In most people's experience, there is typically a dramatic change in facial appearance with GH replacement. This might be partly explained not only by changes in skin texture and thickness but also by a loss of excess subcutaneous fat combined with an increase in tissue hydration.

Hypopituitary adults are usually described as having dry skin, whereas excessive sweating is a typical feature of acromegaly. Eccrine sweat glands have been shown to possess GH receptors.[25] Sweat secretion, measured in response to pilocarpine iontophoresis, was significantly lower in AGHD patients than in age- and sex-matched control subjects; during rhGH treatment, there was a significant increase in the sweat secretion rate that was perceived by the patients.[2] GHD adults have been shown to have an impaired ability to dissipate heat by sweating following heat stress or exercise,[26] and this may be a contributory factor to their reduced exercise capacity.

PHYSICAL PERFORMANCE

Muscle Strength

In our original study, isometric quadriceps force in AGHD was significantly reduced, on average by 26%, compared with matched controls.[27] Most of the deficit could be explained by the reduction in muscle cross-sectional area. However, after correction for this, an additional small deficit was still present in the AGHD patients, possibly explained by a lack of training.

The effects of relatively short-term (up to 6 months) rhGH treatment on muscle strength in AGHD were evaluated in several studies. A significant increase in limb girdle strength was found in our patients; however, quadriceps isometric force did not increase significantly in any of the studies despite clear increases in thigh muscle cross-sectional area. Only with more prolonged rhGH treatment of at least 12 months has a significant increase in quadriceps force been demonstrated; after 3 years, a near normalization of muscle strength can be expected.[28] Measuring muscle strength is not easy, and the failure to demonstrate an effect in many cases is because the variance of the method is too great to be able to demonstrate an effect in the small number of patients included.

Exercise Capacity

Maximal exercise performance in AGHD has been assessed by cycle ergometry. Before treatment, values for maximum oxygen uptake were significantly reduced, being on average 72% to 82% of those predicted for age, sex, and height.[7,29] Following rhGH treatment for up to 6 months, maximum oxygen uptake increased significantly, reaching a mean of 97% of predicted in our patients. Parallel increases in power output and work capacity also have been observed.[30] Submaximal exercise performance representing most closely the exercise taken in normal life, measured as anaerobic threshhold, increased significantly with rhGH treatment, suggesting that physical activities of daily living would be accomplished with less metabolic stress and thus with less subjective sensation of effort. In contrast to the relatively rapid normalization of exercise capacity we observed in patients with AOGHD, data from patients with COGHD suggest that it may take up to 3 years to attain maximum benefit.[31] Possible explanations for the enhanced exercise performance with rhGH treatment include the increase in skeletal muscle mass, increased ECF, improved cardiac function (see below), improved heat dissipation through increased sweating capacity, and as yet undetermined alterations in fuel utilization during exercise.

CARDIAC FUNCTION

Echocardiography in our original study revealed a small but significant increase in resting left ventricular (LV) end-diastolic volume (mean, 2%) and stroke volume (mean, 6%) with rhGH treatment, most likely resulting from an increase in plasma volume (preload) secondary to the antinatriuretic effect of GH.[13] There also was, however, a significant increase in LV wall mass, which occurred in the absence of any change in mean arterial pressure. The mean increase in LV wall mass of 5% was comparable with the 5% to 10% increase in thigh muscle and LBM in the same patients, suggesting an anabolic action of GH on both skeletal and cardiac muscle. In adults with COGHD, cardiac mass was reduced and cardiac function was impaired,[32,33] and both were normalized after 6 months of rhGH treatment, returning to baseline 6

months after treatment stopped.[32] A sustained increase in cardiac output has been observed in AGHD after 3 years of rhGH treatment.[34]

Two remarkable case reports of cardiac cachexia in GHD adults that responded dramatically to GH replacement further emphasize the importance of GH in attaining and maintaining optimal cardiac function.[34-36]

RENAL FUNCTION

Glomerular filtration rate and renal plasma flow were significantly lower in AGHD compared with age-matched healthy individuals.[2] GH replacement resulted in a normalization of both variables and in our patients induced a rapid and marked increase in creatinine clearance and a fall in blood urea.[3] It is not known to what extent these findings reflect changes in plasma volume, cardiac output, kidney size, or other intrarenal effects.

METABOLISM

Energy Expenditure

The anabolic action of GH is associated with an increase in energy expenditure. Basal metabolic rate (BMR) increased by an average of 22% in our patients after 1 month of rhGH treatment and was still 16% higher than baseline after 6 months.[2] The increase in BMR at 6 months could be largely explained by the increase in LBM (known to be the major physiologic determinant of BMR), whereas the mean BMR at 1 month was still significantly raised even when expressed per kilogram of LBM. GH increases the peripheral conversion of thyroxine (T_4) to triiodothyronine (T_3),[2,31] and rhGH treatment in AGHD results in a rise in circulating T_3 levels both in patients on T_4 replacement and those not needing it. This may help to explain the initial increase in BMR. Stimulation of previously underactive metabolic processes, particularly protein synthesis[20,37,38] and fat oxidation,[38-41] also may contribute to the calorigenic effect of GH. From a practical therapeutic aspect, free T_3 levels should be monitored when GH replacement is started, and reduction in T_4 replacement dose may be needed to prevent iatrogenic hyperthyroidism. Plasma thyrotropin and free or total T_4 are of little or no value in monitoring thyroid status in GHD patients on GH replacement.

Carbohydrate Metabolism

The increase in fat mass and its central distribution in AGHD is associated with insulin resistance, reflected in a strong correlation between fat mass and fasting insulin and C-peptide levels in these patients.[42] This contrasts with the increased insulin sensitivity seen in children with GHD.

Following rhGH treatment in AGHD for 1 to 3 months, modest increases have been observed in fasting and postprandial blood glucose concentrations (within the normal range) as well as in fasting serum insulin and C-peptide concentrations.[3,20] This is in keeping with a further increase in insulin resistance, which was confirmed recently using a glucose clamp technique.[43] Glycosylated hemoglobin concentrations, however, have shown no significant changes after up to 3 years of rhGH treatment.[3,8,28] It also is reassuring that glucose, insulin, and C-peptide concentrations and insulin sensitivity assessed by glucose clamp all tend to return towards baseline values by 6 months of rhGH treatment, suggesting an over riding beneficial effect of shedding fat and increasing LBM on insulin action. It will be of interest to see whether insulin sensitivity is eventually normalized with long-term treatment.

Protein Metabolism

Whole-body protein synthesis (assessed by the continuous infusion of C^{13}-leucine) was found to be lower in GHD than in matched controls,[37] as were leucine flux, leucine oxidation, and the incorporation of leucine into protein.

In a double-blind, placebo-controlled study involving 18 patients and using a continuous infusion of C^{13}-leucine, stimulation of protein synthesis was demonstrated after 2 months of rhGH treatment. This remained significant even when results were corrected for the increase in LBM.[38] However, when C^{13}-leucine turnover was examined after a longer period (6 months) of treatment, no increase in leucine flux or protein synthesis was observed,[37] possibly because a new steady state had been achieved.

GH, IGF-1, and insulin act synergistically to promote anabolism during rhGH treatment. GH and IGF-1 stimulate protein synthesis and the accompanying rise in circulating insulin inhibits proteolysis (Figure 3).[44,45]

Lipoprotein Metabolism

In AGHD, mild increases in total and low-density lipoprotein (LDL) cholesterol levels have been observed in up to 50% of patients compared with age-, weight-, and sex-matched controls, in keeping with earlier findings in GHD children.[46,47] GHD adults also tend to have low high-density lipoprotein (HDL) cholesterol levels and high

Figure 3

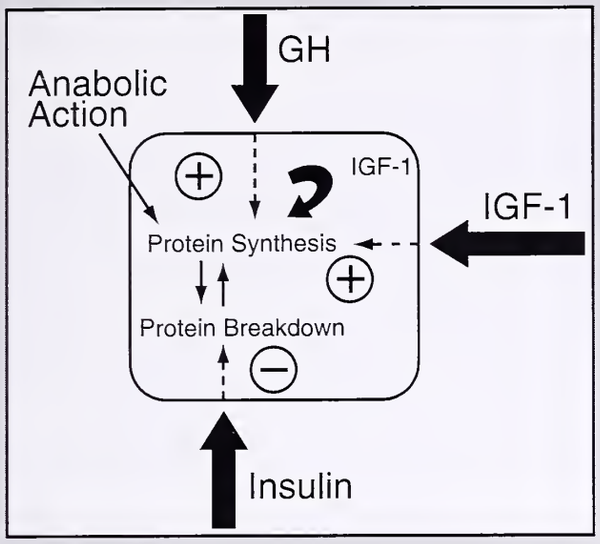

Figure 3. Summary of the interactions among insulin, IGF-1, and GH in the regulation of protein synthesis. GH and IGF-1 stimulate protein synthesis directly while insulin enhances this anabolic action by inhibiting protein breakdown.

triglyceride levels. Following rhGH treatment in AGHD, several studies have demonstrated a significant fall in total and LDL cholesterol concentrations, accompanied by a modest rise in HDL cholesterol and no change in triglyceride levels.[20,47-49] However, data on lipoprotein(a), an independent risk factor for the development of atherosclerosis and myocardial infarction, have been less consistent with either a rise[48] or no change[49] being observed. This may be due to differences in assays, patient selection, or duration of rhGH treatment.

Alterations to the lipid profile during rhGH treatment could occur through direct actions of GH and/or IGF-1 on lipoprotein synthesis or clearance. An indirect effect also is possible via the rise in circulating T_3 levels or the reduction in central adiposity accompanied by an amelioration of the insulin-resistant state.

The clinical relevance of the unfavorable lipid profile of AGHD and its improvement with rhGH treatment is apparent when considered in light of a large retrospective epidemiologic study from Sweden. Overall mortality in 333 hypopituitary patients was found to be almost 2-fold higher than that in an age- and sex-matched normal population, despite adequate thyroid, adrenal, and sex hormone replacement.[1] The increase in overall mortality was largely accounted for by an increase in cardiovascular deaths. It was suggested that GHD, which could be assumed to be present in most of the hypopituitary patients, was a factor in the increased cardiovascular mortality. This proposed association has been strengthened by the demonstration of an increased prevalence of atherosclerosis in hypopituitary adults on full conventional hormone replacement.[50] Very long-term observations will be required to determine if GH replacement in AGHD results in a regression of atherosclerosis and ultimately in a reduction in cardiovascular mortality.

PSYCHOLOGIC WELL-BEING

Detailed assessment of psychologic well-being was performed in AGHD in our initial study, using 3 well-validated but independent generic questionnaires.[51] Responses prior to treatment were compared with those from normal subjects matched for age, gender, ethnic origin, socioeconomic class, and area of residence. The GHD patients perceived themselves as having a poorer quality of life than the normal subjects. Particular areas of concern to the patients were low energy, emotional lability, low mood, and social isolation. They also regarded themselves as having a poorer level of general health, less self-control, less vitality, and more anxiety. Indeed, over one third of the patients scored in the range consistent with a psychiatric disturbance requiring therapy.

It is likely that these findings reflect the GHD state per se and are not simply attributable to the presence of a chronic disorder. In a recent comparison of AGHD with age- and sex-matched diabetic patients using standardized psychiatric rating and diagnostic measures, significantly more of the GHD group were identified as definite psychiatric cases (46% vs 24%), with the most frequent diagnosis being major depression.[52] Moreover, in our original placebo-controlled study, treatment of GHD for 6 months resulted in a substantial improvement in psychologic well-being, with many patients verbally reporting a feeling of increased energy and well-being within a few weeks. The responses to the questionnaires confirmed the clinical impression, with statistically significant improvements in perceived physical health and quality of life, especially in the areas of energy and mood.[51]

It is likely that the increases in LBM and skeletal muscle mass contribute to the sense of improved well-being with rhGH treatment, particularly in the long-term. Other possible mechanisms include improved tissue hydration and perfusion, the

increase in circulating T_3, and direct effects of GH and/or IGF-1 within the central nervous system. There is evidence of a direct central effect, with the demonstration of abnormal sleep patterns in young GHD adults that improve after short-term rhGH treatment.[53] It has been shown that GH replacement penetrates the blood-brain barrier, stimulating a rise in the cerebrospinal fluid ß-endorphin concentration with a simultaneous fall in concentrations of homovanillic acid and vasoactive intestinal polypeptide concentrations.[54]

SIDE EFFECTS

The target dose of rhGH chosen for the initial trials in AGHD was 0.06 to 0.07 IU/kg/d (0.02 to 0.023 mg/kg/d), which is similar to that routinely used in CGHD. Side effects forced early dose reductions in a significant proportion of the patients, and this, together with the finding of elevated circulating IGF-1 levels in some cases, suggested that this dose is supraphysiologic for most adults. Subsequent trials have employed lower rhGH doses. With experience, the average replacement dose has fallen to less than 0.02 IU/kg/d (0.006 mg/kg/d).

Clinical evidence of sodium and water retention following rhGH treatment in AGHD is the most common side effect, reflecting the hormone's potent antinatriuretic action. Weight gain, swelling of the ankles, a sensation of tightness in the hands, or symptoms of carpal tunnel compression frequently occur within days or weeks. These symptoms are often transient or resolve rapidly with dose reduction. In many cases, they can be explained purely through normalization of tissue and extracellular hydration. Blood pressure has not changed significantly with rhGH treatment. Arthralgias involving small or large joints also occur in some patients soon after commencing treatment, but usually there is no evidence of effusion or inflammation and X-ray films show no abnormality. These changes also settle spontaneously with time but occasionally necessitate a dose reduction. They are possibly due to swelling of articular cartilage and reactivation of cartilage growth, since patients not uncommonly liken them to the growing pains of adolescence. With lower starting doses and more gradual titration, the incidence of side effects should continue to decrease.

The main limiting factor to widespread use of rhGH in hormone replacement is now cost. In the climate of restricting health-care costs that exists in most countries, it is not easy to convince funding agencies that GH replacement is worth the high cost.

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Orexins and Orexin Receptors: A Family of Hypothalamic Neuropeptides and G Protein-Coupled Receptors That Regulate Feeding Behavior

Two neural proteins with appetite-stimulating properties, their receptors, and respective genes have been identified and characterized in humans, rats, and mice. They are termed orexins (after the Greek word orexis, which means appetite). Orexin-A is a 33 amino acid peptide (molecular weight, 3.56 kd); orexin-B is a 28 amino acid peptide (molecular weight, 2.94 kd); they are derived from a common gene located on human chromosome 17q21 by alternative splicing. Two orexin receptors also have been found; hOX_1R (425 amino acids) and hOX_2R (444 amino acids), and have structures typical of 7 transmembrane G protein-coupled receptors. They are somewhat homologous to receptors for neuropeptide Y and thyrotropin-releasing hormone.

In the rat brain, orexin is located in the lateral and posterior hypothalamic regions (the "feeding center"), but not in the ventromedial, arcuate, or paraventricular nuclei (the "satiety center"). *Prepro-orexin* mRNA levels were upregulated upon fasting, suggesting a physiologic role for the peptides as mediators in the central feedback mechanism that regulates feeding behavior. Administration of orexin-A or -B into the lateral ventricle of male rats stimulated an immediate increase in food consumption.

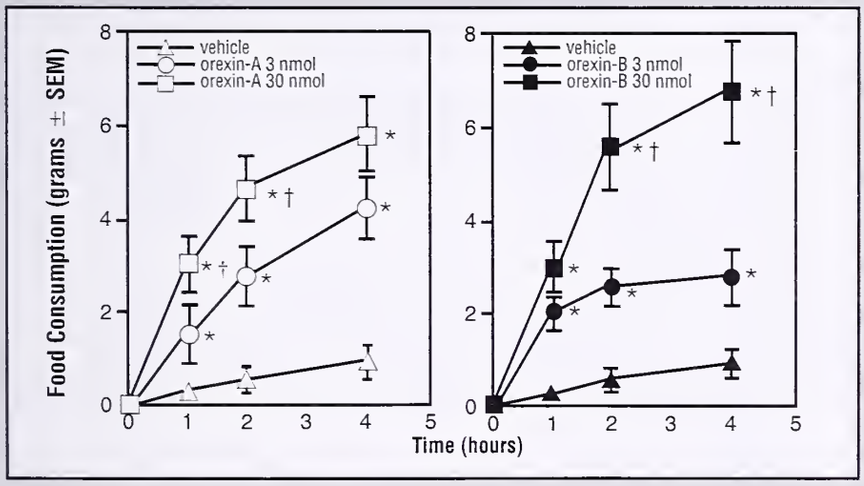
Sakuri T, et al. *Cell* 1998;92:573-585.

Editor's comment: *This interesting finding adds another player to the many that regulate appetite and energy metabolism in humans and animals. Exploration of its interaction with leptin will be of great importance. It is possible that the orexin system may contribute to disorders associated with either hyperphagia or anorexia in humans. The possibility that polymorphic variants of orexin-A or orexin-B and their receptors determine appetite in normal individuals and in patients with feeding disorders will undoubtedly be explored, as will the therapeutic potential of receptor agonists in patients with wasting disorders and of antagonists in obesity.*

The Editorial Board calls to your attention that an excellent review of leptin physiology by Zhang and Leibel appeared in GGH *(1998;14[2]:17-26), which you may wish to read as a foundation for understanding the many articles that are appearing and will appear in the literature concerning appetite and obesity very soon.*

Allen W. Root, MD

Stimulation of Food Consumption by Intracerebroventricular Injection of Orexin-A and -B in Freely-Fed Rats



Designated amounts of synthetic human orexin-A (left) or -B (right) were administered in a 5μ/L bolus through a catheter placed in the left lateral ventricle in early light phase. Cumulative food consumption was plotted over the period of 4 hours after injection. Asterisks (*) indicate significant difference from vehicle controls ($P<0.05$; n = 8-10; ANOVA followed by Student- Newman-Keuls test). Crosses (†) designate significant difference between 3 nmol and 30 nmol injections ($P<0.05$; n = 8-10; ANOVA followed by Student-Newman-Keuls test). Similar results were obtained in at least 4 independent sets of experiments. The same vehicle control curve was replotted in both panels.

Reprinted with permission from Sakurai T, et al. *Cell* 1998;92:573-585.

Further Reports of Leptin Abnormalities in Humans

Montague et al (*Nature* 1997;387:903-908) described the only case reported until now of leptin deficiency. No cases of leptin receptor gene (*LEPR*) deficiency have been described. The 2 articles reviewed here describe the clinical and hormonal consequences of a genetic defect in either the secretion of leptin, the adipocyte hormone that regulates appetite and energy metabolism, or the synthesis of its receptor. Phenotypically, patients with leptin deficiency or insensitivity are morbidly obese and, as adults, hypogonadotropic.

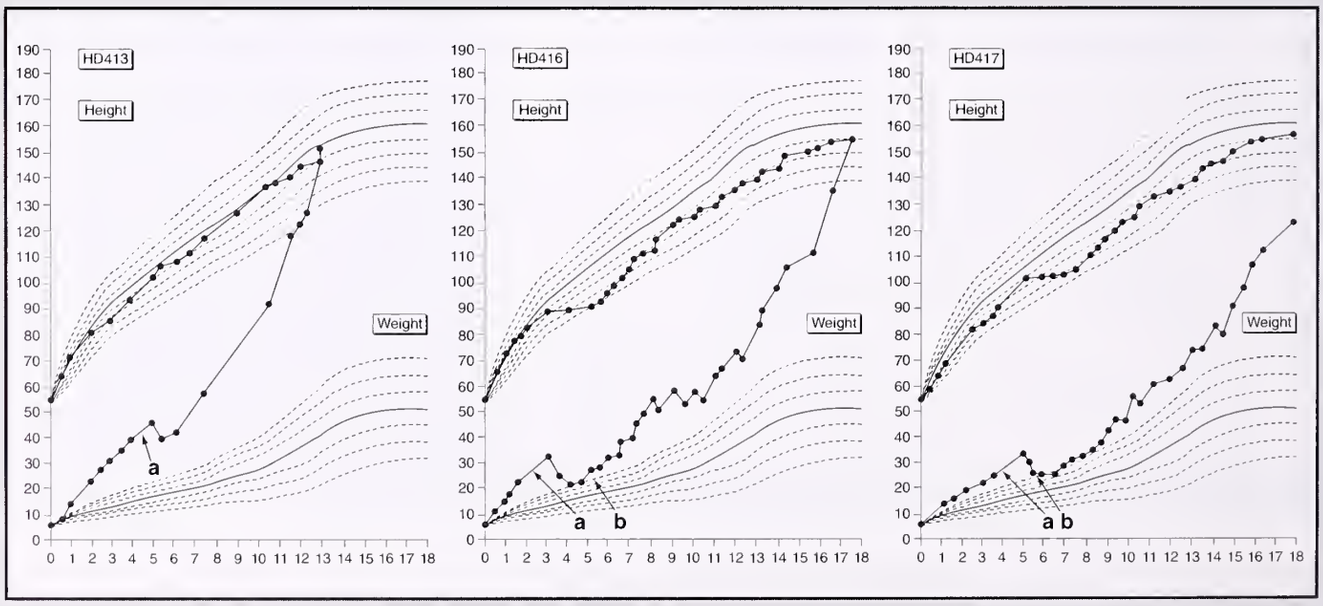
Strobel et al describe a Turkish family in which 3 extremely obese, hyperphagic members had a homozygous C→T substitution in codon 105 (exon 3) of the *OB* (leptin) gene,

leading to replacement of arginine by tryptophan. Body mass index values were markedly elevated (46.9, 55.8, and 32.5) at 34, 22, and 6 years of age, respectively; all had inappropriately low serum leptin levels. Family members who were heterozygous for this mutation were phenotypically normal with normal serum leptin concentrations. The older affected subjects were clinically hypogonadotropic (primary amenorrhea in the 34-year-old female; little virilization in the 22-year-old male). Basal serum concentrations of testosterone were low, but the response to human chorionic gonadotrophin was normal; serum level of luteinizing hormone (LH) was low in relation to the low testosterone. Follicle-stimulating hormone (FSH) was normal, as were the secretory responses of FSH, LH, and testosterone to gonadotropin-releasing hormone. The 22-year-old patient had low sympathetic tone (abnormal cold pressor response and orthostatic hypotension). In transfection studies (COS-1 cells), it was demonstrated that this mutation did not inhibit synthesis of leptin but impaired its processing through the secretory pathway.

Clement et al reported 3 obese female siblings of Kabilian ancestry who had serum concentrations of leptin that were 4-fold higher than the upper limit of normal. They had a homozygous mutation (G→A) in the splice donor site of exon 16, leading to skipping of exon 16 and the synthesis of a leptin receptor (*OB-R*) lacking the transmembrane and intracellular domains of the wild-type *OB-R*. Since this mutation led to secretion of the extracellular domain of the *OB-R* that could dimerize and bind leptin, serum leptin-binding activity was quite high in the homozygous and heterozygous subjects, accounting in part for their elevated serum leptin values. The homozygous mutants had aggressive food-seeking behavior and became obese within the first months of life, similar to subjects with the Prader-Willi syndrome. Growth faltered at approximately 5 to 6 years of age, despite the obesity (Figure). They were found to have subnormal provoked and spontaneous growth hormone (GH) secretion and inappropriately low levels of insulin-like growth factor-1 (IGF-1) and IGF-binding protein 3 (IGFBP-3). During administration of GH, growth rate improved and IGF-1 and IGFBP-3 values rose. The older subjects had primary amenorrhea in the presence of low LH and FSH levels; slightly low free thyroxine values; and perhaps slightly prolonged thyrotropin secretory responses to thyrotropin-releasing hormone, suggesting partial deficiency of this tripeptide. Despite attempts at food restriction, weight gain progressed inexorably; 1 subject died at 19 years of age at a weight of 133 kg; the 2 surviving siblings weighed 159 kg at 13 years and 166 kg at 19 years, respectively.

Strobel A, et al. *Nature Genet* 1998;18:213-215.
Clement K, et al. *Nature* 1998;392:398-401.

Editor's comment: *These articles confirm the essential role of leptin in the regulation of appetite and weight in*



Height and weight curves for the 3 affected sisters from birth to adult age. The letter **a** indicates a period of food and food-intake restriction. The restrictive diet of 500 kcal/d resulted in weight loss and in a dramatic decrease in growth velocity that persisted even after food-intake increase and weight regain. The letter **b** indicates the introduction of treatment with levothyroxine and the start of treatment with exogenous growth hormone. The x-axis indicates age in years; the y-axis indicates height in centimeters or weight in kilograms.

Reprinted with permission from Clément K, et al. *Nature* 1998;392:398-399.

humans and its likely importance in pubertal development. The influence of leptin on sympathetic tone is of interest, although the mechanism by which it exerts this effect is unknown at present. The finding of suboptimal GH secretion in the leptin-resistant subjects is unexpected, as patients with leptin deficiency reported to date have had normal linear growth patterns. The criteria employed for the diagnosis of GH deficiency in the leptin-resistant subjects seem reasonable, although the decline in growth rate appeared to coincide initially with a period of food restriction and weight loss. If the leptin receptor influences GH secretion in humans, it may be through regulation of hypothalamic GH-releasing hormone synthesis or secretion.

Allen W. Root, MD

2nd Editor's comment: *The investigators of both papers are commended for performing very important studies. While the data in the second paper pertaining to obesity, sexual infantilism, hyperinsulinemia, and leptin levels are interpreted correctly, I am reluctant to interpret the auxologic and/or biochemical data as being convincing evidence of GH deficiency. The growth curves are not the growth curves of GH-deficient children. The growth of the 3 affected children was never below the 3rd percentile. Two unaffected children (No. 412 and No. 419) were essentially of comparable heights at essentially the same ages as No. 413 and No. 417, who were affected; the latter 2 had not had sex steroids to enhance their growth. The low GH levels are compatible with those often seen in obese children. The IGF-1 levels are marginally low, but no sex steroids were present to stimulate GH production and, consequently, generation of IGF-1. In my opinion, further observations on similar patients are needed for the argument of GH insufficiency to be convincing.*

Robert M. Blizzard, MD

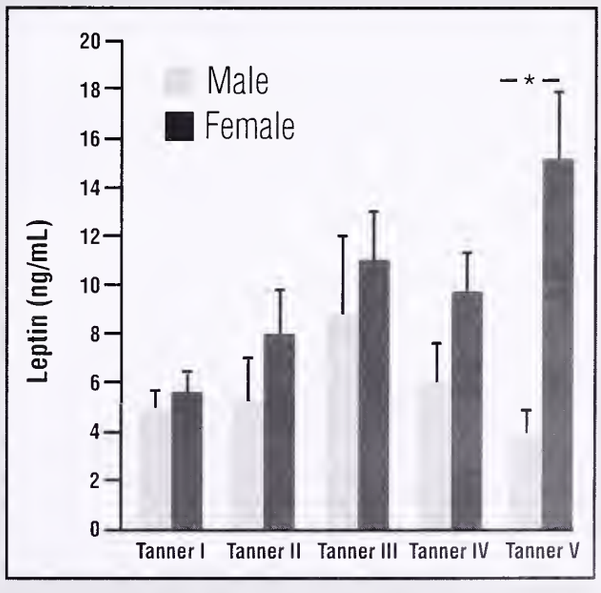
Leptin Plasma Levels in Healthy Spanish Children and Adolescents, Children With Obesity, and Adolescents With Anorexia Nervosa and Bulimia Nervosa

In order to determine normal circulating levels of leptin throughout adolescence as well as in children with eating disorders (obesity, anorexia nervosa, and bulimia nervosa), 100 normal children were prospectively included in this study. They were divided into 5 different groups, corresponding to each of the 5 Tanner stages. These children had height and growth velocities between ±1 SD and body mass index (BMI) within ± 2 SD. Their bone ages were similar to their chronologic ages. These normal subjects were compared with 14 prepubertal obese children with BMI >2 SD. Fasting blood samples were taken in the morning. Leptin levels were measured at 6 and 12 months when 25% and 50% of BMI-SDS reduction was achieved by the obese group, respectively.

Eleven Tanner stage V females with anorexia nervosa also were included in the study. Leptin levels in these anorexic patients were measured after weight gains of 8% to 10% above their original weight. Bound and free leptin levels also were determined in 3 anorexic and 3 bulimic patients.

Significant changes in leptin level were observed throughout puberty at different Tanner stages. Normal males exhibited only 1 peak level at Tanner stage III, while normal females revealed 2 peaks at Tanner stages III and V. Linear correlation between leptin and BMI-SDS was found in lean normal subjects. Mean leptin levels for obese prepubertal children were significantly elevated compared with age- and sex-matched controls. Normal leptin levels were reached after 1 year on a low-calorie diet and weight loss of at least 50% of the initial BMI-SDS. No direct correlation was found between BMI-SDS and leptin levels in the obese group.

Patients with anorexia nervosa exhibited lower plasma leptin levels compared with age- and sex-matched controls. Those patients with bulimia have higher leptin levels than patients with anorexia nervosa and do



Mean circulating leptin plasma levels (± SEM) in healthy Spanish males and females at different stages of sexual maturation. A significant effect of both sex and Tanner stage was found on leptin levels (2-way ANOVA; $P<0.0001$). Circulating leptin levels are significantly sexually dimorphic only at Tanner stage V(*).

Reprinted with permission from Argente J, et al. *J Pediatr* 1997;131:833-838.

not differ significantly from controls. Total and free leptin levels were higher in bulimic patients than in anorexic patients, but no differences were found in levels of the bound form.

Argente J, et al. *J Pediatr* 1997;131:833-838.

Editor's comment: *This paper reports new leptin level data throughout normal developmental stages and its correlation with body weight in 2 different pathologic states, as well as after a phase of weight recovery in obese and anorexic patients. The data presented in this article confirm previous findings during adulthood reported by Ferron et al* (Clin Endocrinol *[Oxford] 1997;46:289).*

The authors also demonstrated differences between different leptin fraction levels in those patients with anorexia and bulimia; however, these values were not compared with those of normal subjects. Previous reports in adult populations already have described the biokinetics of the different leptin fractions in normal and obese subjects. Thus, it would have been interesting to see in this particular study not only how the leptin profiles change throughout the normal development stages but also the kinetics of different leptin fractions in normal subjects throughout childhood. Although determination of leptin values may be helpful to assess adipose tissue stores, it still is not clear what their clinical role is in the diagnosis or prognosis of severe eating disorders.

Zhang et al recently contributed an outstanding lead article concerning leptin physiology in GGH *(1998;14[2]:17-26), which readers will find most enlightening.*

Fima Lifshitz, MD

J Clin Invest 1998;98:1277-1282.
Diabetes 1996;45:1638-1643.

The APECED Gene and Its Products and Polyglandular Autoimmune Disease I

Autoimmune polyglandular syndrome type 1, also termed autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), is a monogenic, autosomal recessive disorder associated primarily with autoimmune hypoadrenalism, hypoparathyroidism, and chronic mucocutaneous candidiasis; it also is associated to a lesser extent with ectodermal dystrophies (vitiligo, alopecia), pernicious anemia, hepatitis, hypogonadism, hypothyroidism, and diabetes mellitus.

By linkage analysis, this disorder has been mapped to chromosome 21q22.3. Two groups of investigators have now isolated and characterized the gene that is mutated in and responsible for APECED.

Since it is likely that the product of this gene is a regulator of transcription of genes associated with immune function, it has been named *AIRE* (autoimmune regulator). It contains 2,027 bp with an open reading frame of 1,763 bp encoding 14 exons; the main transcription product (AIRE-1) has 545 amino acids without a transmembrane or signal peptide, but with a nuclear targeting signal, 2 cysteine-rich zinc-finger DNA-binding domains (amino acid 299-340 and 434-475), a proline-rich region, and 3 LXXLL sequences—domains associated with DNA and protein interaction. Two alternatively spliced products also have been found composed of a separate first exon and exons 8-14: AIRE-2 (348 amino acids) and AIRE-3 (254 amino acids). AIRE-1 is expressed in the thymus, adrenal cortex, and pancreas as well as in the spleen, lymph node, bone marrow, fetal liver, and testis.

Homozygous and compound heterozygous mutations in *AIRE* have been found in Finnish, Swiss, Dutch, and German families with APECED, including 5 mutations resulting in frameshifts and truncated proteins and loss of the zinc-finger domains. No mutations in AIRE-2 or AIRE-3 have been found to date in patients with APECED.

Nagamine K, et al. *Nature Genet* 1997;17:393-398.
Finnish-German APECED Consortium. *Nature Genet* 1997;17:399-403.

Editor's comment: *Autoimmune polyglandular syndrome type 2 (primarily autoimmune hypoadrenalism, diabetes mellitus, and thyroid disease) and other polyglandular syndromes such as pernicious anemia and autoimmune thyroid disease are clearly linked to the HLA system (chromosome 6p) and presumably result from an aberration in immune surveillance related to antigen presentation or recognition. Since APECED is not linked to HLA, the protein product of* AIRE *(which has many characteristics of a regulator of gene transcription) introduces another potential pathway of immune regulation. As the genes regulated by* AIRE *are identified and the biologic functions of the products of these genes determined, our understanding of the mechanisms of immunity and disorders thereof will increase. Parenthetically, it is interesting to note that the Down syndrome critical region also is assigned to chromosome 21q22.3. It is possible that the propensity for autoimmune thyroid disease characteristic of the Down syndrome patient may be related to* AIRE.

Allen W. Root, MD

Efficacy and Safety of Growth Hormone Treatment in Children With Prior Craniopharyngioma: An Analysis of the Pharmacia and Upjohn International Growth Database (KIGS) From 1988 to 1996

This article presents data regarding the use of human growth hormone (hGH) in children with craniopharyngioma. Extensive data (collected from 1988 to 1996) were extracted from the Pharmacia and Upjohn International Growth Database. The database showed that 488 patients had a prior history of craniopharyngioma (280 boys, 208 girls). The modality of treatment of craniopharyngioma was known in 451 cases: 251 were treated with surgery alone; 144 had surgery plus irradiation; 12 received only irradiation; and 44 had received no surgery or radiation. hGH treatment was begun at a median time of 1.56 years (mean, 2.23 $\pm$ 1.88 years) after tumor diagnosis and was given in a mean dose of 0.49 $\pm$ 0.15 IU/kg/wk (0.15 mg) in 3 to 7 injections. Of the group, 40.4% were treated with hGH alone, but others received hydrocortisone and other replacement hormones.

Three hundred ninety-four children completed 1 year of hGH treatment; 152 who were prepubertal at the start of treatment completed 5 years of hGH treatment. The median height SDS increment was 0.9 after 5 years. The gain in height SDS was not influenced by tumor recurrence. Bone age increased 4.5 years in 5 years. Seventy-eight males and 53 females who completed hGH treatment to ultimate height were at a median height SDS of -0.7; 58.8% were above -1 SD in relation to target height. Mean height velocity during the final year of hGH treatment was 4.3 cm/y. Adverse effects included tumor recurrence, with 63 recurrences in 54 patients (11%) after a median of 3.7 years after the initial diagnosis; the longest interval between initial diagnosis and tumor recurrence was 10.3 years.

The authors point out that the response of children with treated craniopharyngioma to exogenous GH was similar to that seen in idiopathic growth hormone deficiency. Growth over 5 years was not influenced by the recurrence of tumor. They also state that they were unaware in every case of the factors involved in the decision to discontinue hGH, but that final height had not been achieved in many of these individuals at that time. Finally, they point out that the recurrence rate of 11% is greater than the rate of 6% to 7% reported in the National Cooperative Growth Study (NCGS) sponsored by Genentech Inc.

Price D, et al. *Hormone Res* 1998;49:91-97.

Editor's comment: *These are important data and help answer the question: "When does one begin GH therapy in children with treated craniopharyngioma?" The individuals reported in this study began their treatment at a mean of 2.3 years after tumor diagnosis. What remains unclear is why the decision was made to begin therapy at that time.*

The authors are correct in pointing out that their recurrence rate is greater than that from the NCGS in the United States for craniopharyngioma (6.4%). The conclusions from NCGS and the current report suggest that exogenous GH does not increase the risk for tumor recurrence.

William L. Clarke, MD

For a complete review of the diagnosis and management of craniopharyngioma, see GGH *1994;10(3):6-10.*

Metabolic Effects of Long-Term Growth Hormone Treatment in Prepubertal Children With Chronic Renal Failure After Kidney Transplantation

Patients included in this report on metabolic data for the German Study Group for Growth Hormone Treatment in Chronic Renal Failure (CRF) had a height SDS of $\leq$-2.0 and/or a height velocity <25th percentile, a glomerular filtration rate (GFR) of <60 mL/min/1.73 m^2 in conservatively treated patients, and a GFR >20 mL/min/1.73 m^2 in patients after renal transplantations (RT). Fifty-three children were prepubertal at the start of recombinant human growth hormone (rhGH) therapy and remained prepubertal throughout the observation period. Twenty-nine of the patients were on conservative treatment for CRF, 14 patients were on dialysis, and 10 other patients had functioning renal allografts. All were on immunosuppressant therapy with cyclosporine, azathioprine, and methylpred-nisolone.

Twelve healthy prepubertal children being evaluated for idiopathic short stature formed the control group. None had rhGH deficiency but had received rhGH therapy. The CRF patients received rhGH at a dose of 28 to 30 IU/m^2/d (0.31 to 0.33 mg/kg/d). Control subjects received rhGH 24 IU/m^2/d (0.26 mg/kg/d). Biochemical examinations included Hb_{A1c}, GFR, and a standard oral glucose tolerance test (OGTT), including insulin values.

Prior to administration of rhGH, Hb_{A1c} and glucose responses during the OGTT were significantly elevated in all patient groups compared with controls. Fasting and integrated glucose concentrations were significantly higher in dialyzed patients than in those treated conservatively or those with RT. As anticipated in RT patients, the fasting 2-hour postprandial glucose was

positively correlated with the daily corticosteroid doses. Fasting serum insulin levels were elevated in the renal failure patients, with the highest levels being in the posttransplant group.

Fasting and OGTT glucose responses did not change throughout the observation period. However, fasting and stimulated insulin levels were 2-fold increased compared with baseline after the first year of rhGH therapy in the dialysis and RT patients, as well as in the controls. Insulin levels in the conservatively treated group became significantly elevated only after the second treatment year. Four patients, 2 on conservative treatment, 1 on dialysis, and 1 RT recipient, developed transient impairment of oral glucose tolerance as defined by the National Diabetes Group of the National Institutes of Health.

In conclusion, the authors observed a selective increase in fasting and glucose-stimulated insulin secretion without a change in glucose tolerance in patients with CRF after RT, but also in short normal children in response to rhGH therapy. This phenomenon was exaggerated in patients on dialysis and after RT, and persisted for up to 5 years of rhGH treatment. Although the absence of increased glucose intolerance during long-term rhGH treatment is reassuring with respect to the diabetogenic potential of rhGH, the persisting hyperinsulinemia, combined with the dyslipidemia associated with CRF, raises concerns that rhGH therapy may contribute to the long-term risk for premature atherosclerosis in patients with childhood-onset CRF.

Haffner D, et al. *Pediatrics* 1998;43:209-215.

Editor's comment: *This interesting study demonstrates that the effects of rhGH on glucose-stimulated insulin secretion are not different for children with CRF and those with idiopathic short stature. The authors point out that associated hyperinsulinemia may be of particular concern in children with uremia and other factors, including dyslipidemia, which may contribute to atherosclerosis. These data are compatible with those reported on US patients and summarized in* GGH *(1996;12[4]:49-53) by Dr. Richard Fine. He points out in his review that there have been no clinical consequences associated with the hyperinsulinemia, as corroborated by the German Study Group. However, the long-term effect of such treatment remains to be shown.*

William L. Clarke, MD

Familial Hyperinsulinism Caused by an Activating Glucokinase Mutation

Hyperinsulinism (HI) is relatively common. It is the most common cause of hypoglycemia. Affected individuals are at risk for seizures and permanent brain damage. Glaser et al describe a family with HI associated with a mutation in the glucokinase gene. Glucokinase is an enzyme with low affinity for glucose that controls the rate-limiting step of beta-cell glucose metabolism.

A mutation of the glucokinase gene has been detected in a 31-year-old white male, his 2 children, his sister, and his father. The mutation was sought in the proband after he became unconscious with low plasma glucose (38 mg/dL) and elevated insulin levels. Counter regulatory hormone responses were normal, and so were pancreatic CT and MRI findings. The proband's 2 children had hypoglycemic seizures and also were diagnosed with HI. Urinary amino acids and urinary and plasma carnitines were normal, as was a pancreatic ultrasound. The proband's sister was diagnosed as having hypoglycemia at the age of 15 years; she had low fasting blood glucose and high plasma insulin levels, and later developed multiple sclerosis. Oral glucose tolerance tests in the proband and his sister showed hypoglycemia 3 hours after taking glucose. During hypoglycemia, their plasma insulin and C-peptide concentrations were elevated. In the proband and his sister, exogenous insulin administration resulted in a decrease in plasma glucose and plasma C-peptide concentrations. The proband's sister's children were normal. The proband's father had symptoms of hypoglycemia, which were controlled by diet. At the age of 48 years, the father developed insulin-dependent diabetes mellitus. All the affected family members were treated with diazoxide 100 to 300 mg/d. This same mutation was not detected in 37 unrelated white families, including 6 with an apparently autosomal dominant form of hyperinsulinism.

The *Val455Met* mutation in the glucokinase gene results in an increased affinity of glucokinase for glucose, resulting in a higher rate of glucolysis and therefore a higher rate of insulin secretion. This represents a

clinically and biochemically distinct autosomal dominant form of familial hyperinsulinism.

Glaser B, et al. *N Engl J Med* 1998;338:226-230.

Editor's comment: *Identification of this mutation in the glucokinase protein is another step in understanding glucose homeostasis. It is clear that different mutations within the same gene give rise to different phenotypes requiring different therapies. The* Val203Ala *mutation within the same gene results in loss of function and gives rise to maturity-onset diabetes mellitus. That particular glucokinase mutation has been identified in about 50% of individuals with gestational diabetes. By contrast, this new type of mutation leads to hyperinsulinism and hypoglycemia. This contrast illustrates that the domain of mutations within a gene can lead to striking differences in phenotypes.*

Judith G. Hall, MD

2nd Editor's comment: *Most patients with familial HI have a defect in the sulfonylurea protein resulting from a* SUR *gene mutation. An excellent article to review in conjunction with this article is by Permutt et al, entitled "FHI: An Inherited Disorder of Spontaneous Hypoglycemia in Neonates and Infants"* (Diabetes Reviews *1996;4:347-353*). *Permutt et al provide the foundation to better understand the etiologies and variations of familial HI, previously called leucine-sensitive hypoglycemia and/or nesidioblastosis.*

Robert M. Blizzard, MD

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Births After Intracytoplasmic Injection of Sperm Obtained by Testicular Extraction From Men With Nonmosaic Klinefelter's Syndrome

Klinefelter's syndrome results from the presence of an extra X chromosome (47,XXY) in males. It is a relatively common sex chromosomal abnormality, occurring in about 1 in 500 males. Some individuals with Klinefelter's syndrome are mosaics, ie, they have both 46,XY and 47,XXY cells. Individuals who are mosaic (46,XY/47,XXY) may have some degree of spermatogenesis and may be fertile, compared with nonmosaic Klinefelter men (47,XXY), who typically have azoospermia and infertility.

Palermo et al have reported 2 couples in which the nonmosaic Klinefelter's syndrome males had undergone testicular sperm extraction (followed by in vitro fertilization by intracytoplasmic injection of single sperm) and thereby were able to father healthy newborn infants.

In the case reports, the men were 32 years and 34 years old and their wives were 32 and 33 years old. Both women were healthy and normal, while both men had nonmosaic Klinefelter's syndrome (47,XXY). Both men had gynecoid habitus, gynecomastia, and bilateral atrophic testes. The first man had bilateral varicocele; the second man had a moderate-size left varicocele. Both men had high serum gonadotropin and low serum testosterone levels. Both men had only Sertoli cells on testicular biopsies. Three semen analyses of the first man showed normal volumes and fructose and a single abnormal nonmotile sperm in 1 semen specimen. Analysis of the 3 semen samples from the second man revealed low volume, normal fructose, and no sperm.

Both women were given leuprolide (a gonadotropin-releasing hormone agonist) subcutaneously to inhibit gonadotropin secretion and then a combination of human menopausal gonadotropin and follicle-stimulating hormone intramuscularly. Oocytes (15 to 40) were retrieved by ultrasonographically guided transvaginal needle aspiration after intramuscular administration of chorionic gonadotropins.

Simultaneous testicular biopsies were performed in the men. Both men had received testolactone 3 months before having a testicular biopsy. After intracytoplasmic sperm injection and fertilization of oocytes, embryos (3 for each woman) were selected and transferred. Both couples refused preimplantation diagnosis.

Both women received daily intramuscular injections of 50 mg progesterone in oil until fetal heartbeats were confirmed by ultrasound. The ultrasound of the first woman at 32 days of embryo transfer revealed 2 asymmetric uterine sacs, only one of which had a fetal heartbeat. Ultrasound of the second woman showed 2 intrauterine sacs, both with fetal heartbeats. Amniocentesis at 20 weeks showed a fetal karyotype of 46,XY in the first pregnancy and fetal karyotype of 46,XX and 46,XY in the second pregnancy.

A healthy boy was born to the first couple; he had a birth weight of 2,778 g at 38.5 weeks gestation. Two healthy children, a 2,551-g boy and a 2,410-g girl, were born by cesarean section to the second couple.

Palermo G, et al. *N Engl J Med* 1998;338:588-590.

Editor's comment: *Assisted reproductive technology has become increasingly important and has revolutionized the treatment of infertility. Preimplantaion diagnosis is now possible in which DNA can be analyzed from a single blastomere. This allows selection of disease-free embryos for transfer to the uterus. In the pregnancies described above, the parents chose to take no risk of loss at that stage, but opted for more conventional prenatal diagnosis by amniocentesis. Intracytoplasmic sperm injection is a relatively new development in assisted technology and provides new hope for couples in whom in vitro fertilization has failed or when there is paucity of viable sperm. This technique is quite promising for nonmosaic Klinefelter's syndrome men, who may now be able to have their own biologic children through this new technology. Interestingly, the number of men recognized to be infertile has been increasing for more than a decade. Precise diagnosis of male infertility will help to provide options to couples.*

Judith G. Hall, MD

Screening for Retinopathy in the Pediatric Patient With Type 1 Diabetes Mellitus

Diabetic retinopathy is the leading cause of blindness in the United States, in patients between the ages of 20 to 74 years. Individuals with type 1 diabetes mellitus are at a high risk for developing diabetic retinopathy. The American Academy of Pediatrics has recently published a statement regarding recommendations for ophthalmologic evaluation of asymptomatic children with type 1 diabetes mellitus.

The statement provides background about diabetic retinopathy and the rationale for the ophthalmologic examination for diabetic retinopathy. An examination schedule for diabetic retinopathy for asymptomatic individuals with type 1 diabetes mellitus also is suggested (Table).

The objective and goals of the statement are to (1) develop a program for assessing children with type 1 diabetes mellitus on a regular basis to prevent diabetic retinopathy as part of the diabetic management; (2) identify children who may be at risk for developing diabetic retinopathy; (3) refer patients appropriately for ophthalmologic examination; and (4) educate individuals with diabetes and their families regarding the benefits of good diabetic control. The members of the committee believe referral to an ophthalmologist for follow-up is essential because ophthalmologists are much better able to detect early retinopathy than primary care physicians. HMOs often are reluctant to refer patients to ophthalmologists for such exams, and this poor practice is unacceptable.

Sections of Endocrinology and Ophthalmology American Academy of Pediatrics. *Pediatrics* 1998;101:313-314.

Editor's comment: *The American Academy of Pediatrics guidelines will be useful for pediatricians and pediatric endocrinologists taking care of children with type 1 diabetes mellitus. Good control of diabetes mellitus is a first step in preventing diabetic retinopathy. Prompt laser eye surgery can prevent further visual deterioration and delay the onset of blindness as a result of diabetic retinopathy.*

Judith G Hall, MD

Suggested Ophthalmologic Examination Schedule for Asymptomatic Pediatric Patient With Type 1 Diabetes

INITIAL DISCUSSION
Within the first year after diagnosis, child and/or parents should receive counseling by a pediatrician or pediatric endocrinologist regarding the need for ophthalmologic examination and early intervention.

INITIAL EXAMINATION BY AN OPHTHALMOLOGIST *
3 to 5 years after diagnosis if >9 years of age

FOLLOW-UP EXAMINATION †
Annually

DURING PREGNANCY
During first trimester, then every 3 months until delivery

* Poor control or deterioration may indicate an earlier initial examination. An ophthalmologic examination also should be performed in poorly controlled patients before intensification of therapy.

† Abnormal findings will dictate more frequent follow-up examinations.

Partial Hormone Resistance in Mice With Disruption of the Steroid Receptor Coactivator (SRC-1) Gene

The authors demonstrate that in mice inactivation of the steroid receptor coactivator (*Src-1*) leads to decreased growth of the gonads and sex hormone-dependent structures (uterus, prostate) but does not impair fertility. *Src-1* influences gene transcription by increasing histone acetyltransferase activity and other mechanisms, thus enhancing receptor-mediated nuclear gene transcription. The investigators inactivated *Src-1* by deleting ~9 kb of its genomic sequence (446 amino acids) from embryonic stems and then inserting these cells into blastocysts of a strain of C57 mice, thus generating chimeric founders. They then bred heterozygous and homozygous *Src-1*-deficient mutant animals. Heterozygous animals were normal. Homozygous *Src-1*-deficient animals were phenotypically normal, but responded subnormally to several steroid hormones. Uterine growth in response to estrogen was significantly attenuated when compared with normal animals, as was testosterone-induced prostate growth. Testicular size was decreased and breast development impaired in homozygous mutants. Serum concentrations of estradiol and testosterone were slightly elevated in the *Src-1*$^{-/-}$ animals. Surprisingly, fertility was normal. In part, the defect in *Src-1* expression was compensated for by increased synthesis of related steroid receptor coactivators such as *TIF2*. The writers concluded that *Src-1* is important for efficient steroid hormone action in vivo.

Xu J, et al. *Science* 1998;279:1922-1925.

Editor's comment: *Nuclear estrogen receptor (ER)-associated proteins help mediate the transcription-activating effects of the estrogen-ER complex.[1] Flanking the DNA binding domain (DBD) of the ER are 2 independent transcription-activating domains (AF-1 on the amino terminal side of the DBD and AF-2 on the carboxyl terminal side of the DNB, overlapping but distinct from the hormone binding domain). Transcription activated through the AF-2 site is mediated by several coactivating proteins that bind to the AF-2 site after the change in conformation of the ER that accompanies its binding to ligand estrogen occurs.[1] One wonders how soon it will be until patients with partial insensitivity to steroid hormones due to inactivating mutations of steroid receptor coactivating proteins are identified clinically and genomically.*

Allen W. Root, MD

Halachmi S, et al. *Science* 1994;264:1455-1458.

2nd Editor's comment: *Could these ER-associated proteins or similar augmentors contribute to the variations in breast size among women of similar body size and/or adiposity?*

Robert M. Blizzard, M.D.

Of Fingers, Toes, and Penises*

Hox genes encode DNA-binding transcription factors that regulate and coordinate the relative positioning of structures during embryologic development.[1] There are 4 *Hox* complexes in vertebrates. Experimental mutations in *Hox* genes alter the position of the regulated skeletal structures (limbs, digits) as well as their size. Kondo et al developed mice with compound mutations in different *Hox* genes: (1) the hypodactyly allele, which is a deletion of *Hoxa-13*; and (2) null alleles in *Hoxa-13* or *Hoxd-11, -12,* and *-13.* These compound mutant mice had total digital agenesis and agenesis of the genital eminence with absence of the penis in male animals and absence of the bladder and urethra in females. The authors concluded that *Hoxa* and *Hoxd* genes regulate development of the morphogenetic ends of the body—digits at the ends of limbs and genital structures at the end of the trunk.

Kondo T, et al. *Nature* 1997;390:29.

*Capital letters *(HOX, HOXa, HOXd)* designate human genes; small letters *(Hox, Hoxa, Hoxd)* represent animal genes.

Editor's comment: *These experimental findings shed light on the genetic mechanisms that are aberrant in clinical human malformation syndromes involving the* HOX *genes.1 Mutations in* HOXd-13 *are associated with synpolydactyly, an autosomal dominant disorder characterized by duplication of the 3rd and 4th fingers and syndactyly of the 3rd, 4th, and/or 5th toes. The mutation in* HOXd-13 *(chromosome 2q31) involves expansion of a sequence of 15 consecutive alanine residues in the N-terminal region of the normal gene to one with 22 to 29 alanine residues and is thought to be associated with gain-of-function of this protein. A dominant-negative mutation (deletion of 20 amino acids necessary for DNA binding) in* HOXa-13 *(chromosome 7p14-p15) has been associated with the hand-foot-genital syndrome, a disorder characterized by hypoplasia of the thumbs and great toes, clinodactyly of the 5th fingers, abnormalities of the carpal and tarsal bones, penile hypospadias in males, and abnormal urethras and ureters and malformed uteri in females. It is likely that mutations in 1 or more* HOX *genes may be found in patients with minor skeletal malformations (clinodactyly, brachydactyly, brachymetacarpia, brachymetatarsia).*

Allen W. Root, MD

Innis JW. *Curr Opin Pediatr* 1997;9:617-622.

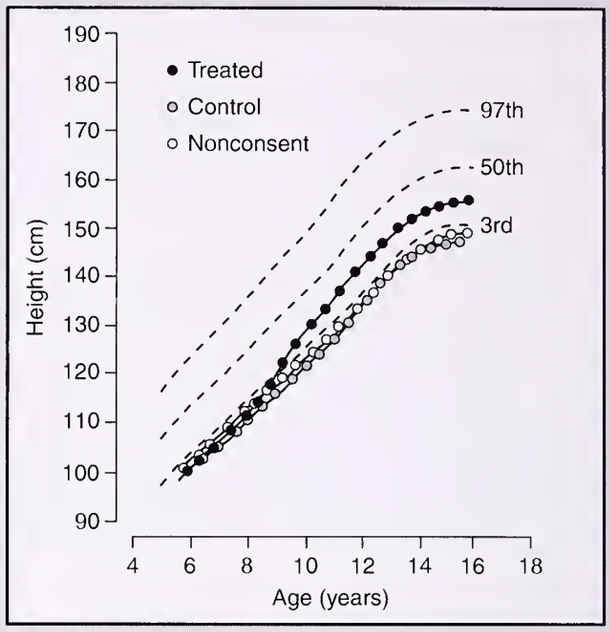
Randomized Trial of Growth Hormone in Short Normal Girls

In a randomized study, the investigators administered recombinant human growth hormone (rhGH) (30 IU/m²/wk [0.33 mg/kg/wk]) to 7 normal short girls (height more than 2 SD below mean height for age) for an average of 6.2 years each. They compared the growth of these children to that of 6 girls who were randomized to a control, untreated group and 19 girls who did not consent to a randomized selection process.

The authors found (Figure) that at near-final height (chronologic age, 16 years) the rhGH-treated subjects were: (1) substantially taller (155.3 cm) than either untreated group (randomized, 147.8 cm; nonrandomized, 149.3 cm); (2) all within their target height range; and (3) 3.5 cm taller than their pretreatment predicted height (whereas the other groups were a mean of 5.5 cm below predicted height). Neither bone age nor puberty advanced more rapidly in rhGH-treated subjects than in the other groups. The increased stature in the rhGH-treated subjects was realized before the onset of adolescent maturation. The authors concluded that rhGH increased final height in normal short girls without affecting the timing or rate of progression of puberty.

McCaughey ES, et al. *Lancet* 1998;351:940-944.

Mean Growth Patterns for Treated Girls and Controls



Reprinted with permission from McCaughey E, et al. *Lancet* 1998;351:940-944.

Editor's comment: *The majority of studies of the effectiveness of rhGH in increasing height in short normal subjects have been performed in males and have been disappointing.[1] McCaughey et al now report that rhGH can increase adult stature in females. Buchlis et al[2] also report that the adult height of short females receiving rhGH was 6.8 cm greater than that of (historical) control subjects, while adult stature of rhGH-treated males was only 3.0 cm greater than that of (untreated) control males. These observations in small groups of short females are tantalizing. Coupled with the observation that rhGH increases adult stature in patients with Turner syndrome,[3] one wonders if the genes on the Y chromosome that influence growth and program the taller stature of males somehow inhibit the growth-promoting effects of exogenous GH in this sex.[4,5]*

Allen W. Root, MD

Guyda HJ. *Trends Endocrinol Metab* 1994;5:334-340.
Buchlis JG, et al. *J Clin Endocrinol Metab* In press.
Rosenfeld R, et al. *J Pediatr* 1998;132:319-324.
Ogata T, Matsuo N. *J Med Genet* 1997;34:323-325.
Lahn BT, Page DC. *Science* 1997;278:675-680.

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

Mutations in *PROP1* Cause Familial Combined Pituitary Hormone Deficiency

The investigators identified 4 families with combined pituitary hormone deficiency due to homozygous or compound heterozygous inactivating mutations of *PROP1*, the "prophet of *Pit-1*," a transcription factor necessary for expression of *POU1F1* (the human homologue of mouse *Pit1*. *POU1F1* is essential for differentiation of the somatotrope, lactotrope, and thyrotrope. Its deficiency results in hypoplasia of the pituitary gland and subnormal secretion of growth hormone (GH), prolactin (PRL), and thyrotropin. *PROP1* has 3 exons encoding a 226 amino acid paired-like homeodomain protein with DNA binding properties. Each of the 3 mutant *PROP1* genes resulted in decreased DNA binding of the product and, hence, to decreased transactivation of a reporter gene. Patients with mutations of *PROP1* lacked not only GH, PRL, and thyrotropin secretion but also luteinizing hormone and follicle-stimulating hormone as well, and were sexually immature. Magnetic resonance imaging revealed hypoplastic pituitaries. The authors concluded that *PROP1* is important for differentiation of gonadotropes and, through expression of *POU1F1*, of somatotropes, lactotropes, and thyrotropes.

Wu W, et al. *Nature Genet* 1998;18:147-149.

Editor's comment: *These elegant studies identify yet another gene necessary for differentiation of the anterior pituitary that now includes* Lhx3 *(LIM homeodomain Zn-binding transcription factor),* POU1F1, *GH-releasing factor, and its receptor.*

Allen W. Root, MD

Frequency of Inherited Bleeding Disorders in Women With Menorrhagia

Menorrhagia is a common gynecologic problem and accounts for 12% of referrals to gynecologists. It involves abnormal uterine bleeding occurring at regular intervals that is excessive in amount and duration. Adolescent girls may have excessive uterine bleeding as they establish their menstrual periods; however, if it is persistent, then a gynecologic evaluation is warranted. There are many etiologies. However, if the pelvic examination is normal, then genetic bleeding disorders should be considered.

Kadir et al screened 150 women with menorrhagia in order to find out what proportion of women with menorrhagia have a genetically related bleeding disorder. Uterine blood loss was assessed by means of a pictorial blood assessment chart. The following were determined for each woman: full blood count, blood grouping, activated partial thromboplastin time, factor VIII activity, von Willebrand factor antigen activity, and factor XI levels.

The authors found that 26/150 (17%) women with menorrhagia who had a normal pelvic examination had a genetically related bleeding disorder: 15/26 had mild von Willebrand's disease; 3/26 had moderate to severe von Willebrand's disease; 4/26 had mild factor XI deficiency; 1/26 had mild von Willebrand's disease and factor XI deficiency; 1/26 had combined von Willebrand's disease, factor XI deficiency, and factor X deficiency; 1/26 was a carrier of hemophilia A; and 1/26 had platelet dysfunction. Overall, 13% had von Willebrand's disease and 4% had factor XI deficiency. Menorrhagia since menarche was noted in 11/123 women (8.9%) without a bleeding disorder, 13/20 women (65%) with von Willebrand's disease, and 4/6 (66.7%) with factor XI deficiency. Women with von Willebrand's disease and factor XI deficiency had prolonged activated partial thromboplastin time. They found that individuals with von Willebrand's disease had a history of easy bruising, bleeding after tooth extraction, postpartum hemorrhage, and postoperative bleeding.

The authors suggest that clinicians treating individuals with menorrhagia should take a careful medical history and test for inherited bleeding disorders, especially von Willebrand's disease. More than 50% of the affected women would have been missed if screening had been done only on the basis of symptoms.

Kadir RA, et al. *Lancet* 1998;351:485-489.

Editor's comment: *Kadir et al found that 1 in 6 women presenting with menorrhagia and a normal pelvic exam has a hereditary bleeding disorder. They point out that they may have a selected population. Nevertheless, the diagnosis of a hereditary bleeding disorder has important implications for prenatal diagnosis, genetic counseling, and future invasive procedures for the affected woman. Because von Willebrand's disease is so common (1.4% of the population), it seems likely that cases of menorrhagia that may be due to von Willebrand's disease are being missed. Although the incidence of bleeding disorders in adolescent females will be less than in adult women, some cases of bleeding disorders—particularly von Willebrand's disease—are being missed. Endocrinologists and gynecologists should be aware of this possibility and avoid it by taking a thorough history and screening for the disorder.*

Judith G. Hall, MD

Post-Program Self-Assessment/CME Verification

Instructions: The Post-Program Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. Which of the following are reduced in hypopituitarism in adults?

   a. skin thickness
   b. total skin collagen
   c. LBM
   d. BMI

2. The use of GH in AGHD may not lead to increased bone density for a year or more.

   a. true b. false

3. The use of hGH Rx has been shown to be psychologically beneficial in >90% of adults with GHD.

   a. true b. false

4. hGH is unknown to:

   a. increase anabolism
   b. increase energy expenditure
   c. increase peripheral conversion of T_4 to T_3
   d. increase fat oxidation
   e. increase insulin sensitivity to CGHD
   f. increase IGF-1
   g. decrease GFR

Answer Key: 1. a, b, c 2. a. 3. b. 4. a, b, c, d, e, f

CME Accreditation Statement

The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education activities for physicians.

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The University of Virginia School of Medicine designates this educational activity for 1.0 hour in Category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Disclosure: As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Sönksen and Weissberger report no conflicts. Drs. Lifshitz, Clarke, Horton, Hall, Rosenfeld, and Slyper report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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